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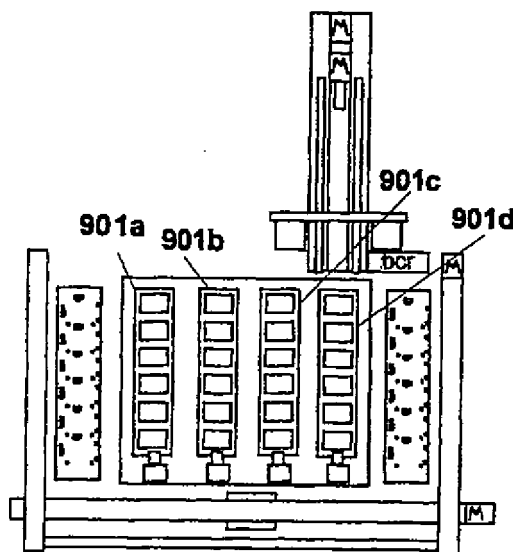
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(54) Title: **AUTOMATED STORAGE AND RETRIEVAL DEVICE AND METHOD**

(57) Abstract: A device and method for the automated storage and retrieval of trays (901a) holding subject matter. A plurality of trays (901a) are inserted into an access device. A computer system is programmed to control a storage gantry to move the trays (901a) between the access device, a storage rack and a work cell gantry.



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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## Automated Storage and Retrieval Device and Method

The present invention relates to automated storage and retrieval devices and methods for using, and in particular to such devices and methods used in conjunction with inspection devices for sequentially inspecting microscopic crystals.

## BACKGROUND OF THE INVENTION

The determination of the three dimensional atomic structure of matter is one of the most important areas of pure and applied research. One way in which the three dimensional atomic structure of matter can be determined is through X-ray crystallography. X-ray crystallography utilizes the diffraction of X-rays from crystals in order to determine the precise arrangement of atoms within the crystal. The result may reveal the atomic structure of substances such as metal alloys, deoxyribonucleic acid (DNA), or the structure of proteins.

There are very important benefits to knowing the accurate molecular structure of a protein crystal. For example, once the molecular structure is known, a drug designer can more effectively develop effective therapeutic agents and drugs. However, despite its promises, X-ray crystallography is limited by the fact that it is very difficult to grow successful crystals.

## Prior Art Method of Growing Crystals

Protein crystals are commonly grown in the wells of micro-well plates. A micro-well plate is also known as a micro-titer plate or a microplate. Micro-well plates typically come with either 24, 48, 96, 384 or 1536 wells. A 96-well micro-well plate is shown in detail in FIG. 2. There are a variety of methods in which protein crystals may be grown. Five common ways are summarized below.

## Hanging Drop Method

One of the main techniques available for growing crystals, known as the hanging-drop or vapor diffusion method, is a method wherein a drop of a solution containing protein is applied to a glass cover slip and placed upside down in an apparatus such as a vapor diffusion chamber where conditions lead to supersaturation in the protein drop and the initiation of precipitation of the protein crystal.

### Sitting Drop Method

Another method is the sitting drop method where the drop sits in a small well adjacent the growing solution instead of hanging over it. This method provides a more stable drop and location.

### Aqueous Drop in Oil Method

Another method is the aqueous drop in oil method. The drop is placed in a micro-well and is covered with an oil based solution. The drop stays at the bottom of the well as the crystal grows.

### Dialysis Method

In another method referred to as the dialysis method (also called microbatch crystallization), the protein solution is contained within a semi-permeable size exclusion membrane and then placed in a solution of fixed pH and precipitant concentration. As the precipitant diffuses through the membrane into the protein compartment, the solubility of the protein is reduced and crystals may form.

### Gel Crystal Growth Method

This method involves the placement of a gel into the end of small diameter glass capillaries. After the solutions have gelled, a protein solution is placed into one end (top) of the capillary and the other end is submerged in a solution of precipitating agent. If the conditions are appropriately selected, crystal growth occurs at a point in the gel where the protein and precipitating agent reach the proper concentrations as the solutions slowly mix by diffusion. Since this is a diffusion limited process, it thus only occurs after an extended period of time. Crystals however, grown by this method are often larger and of higher quality.

Regardless of the method chosen, protein crystal growth is a very delicate and time-consuming process. It can take several days to several months before crystals of sufficient size and quality are grown and ready for x-ray crystallography. The current minimum size that is typically stated is a crystal of at least 50 microns thick by 100 microns in extent. The protein crystal growing environmental conditions need to be rigorously maintained, from the chemistry, to the surrounding air humidity and

temperature, cleanliness to prevent contamination, and even lighting conditions. A protein crystallographer working with unknown protein families may only be about 5% successful in growing proper sized quality crystals. With this success rate, for example, a 96-well micro-well plate may only have 5 wells in which good crystals are growing.

#### Prior Art Inspection of Crystal Growth

Currently, a laboratory technician, or operator, aided by a microscope and a laboratory notebook manually inspects crystals grown in micro-well plates. To inspect a micro-well plate, a laboratory technician dons a clean-room gown suit and enters a cold room in which the crystals are growing. The technician then puts a micro-well plate underneath the microscope and examines each well in the micro-well plate until all of the wells in the micro-well plate have been inspected. The technician then makes a mental judgement as to how he shall classify (also known as "score") the crystal. For example, the technician may feel that he is observing an image that shows "grainy precipitation" or "ugly precipitation". Or, he may feel that the image shows "no crystal growth". The technician then records the classification into a laboratory notebook.

The above system is riddled with opportunities for human error. An operator, manually inspecting a 96-well micro-well plate will take approximately 5 to 20 minutes depending on the skill of the operator and the number of wells that contain interesting features, microcrystals, or crystals. The operator may be subject to physical fatigue, suffer eyestrain, and may be uncomfortably cold in the temperature controlled and generally high humidity room. The operator can be tired and confused and can easily make errors in manually recording data in the notebook. For example, the operator may observe crystal growth at well H5 (FIG. 2), but incorrectly record in the notebook that the crystal growth was at well H6. Additional transcription errors may occur when the data is transferred to a computer database.

Research efforts are underway to try to solve the above problem, but they are inadequate for the needs of the industry. One such effort is described in Jurisica et al. "Intelligent Decision Support for Protein Crystal Growth" *IBM systems Journal*, Vol. 40, No 2, 2001. Another such effort is described at the Website [www.dsitech.com](http://www.dsitech.com).

### Current Problems with Micro-well plate Storage and Retrieval Procedures

Typically, after a technician has inspected a micro-well plate for crystal growth, the micro-well plate is stored until it is time to inspect it again. The growing of protein crystals in micro-well plates and the accompanying inspection of the micro-well plates for successful crystal growth are procedures that are typically carried out concurrently in large quantities in laboratories. For example, a typical lab at any given moment may have literally thousands of micro-well plates in which protein crystals are attempting to grow. The growth cycle of a protein crystal can be approximately 6 months. During the 6 month time period, a micro-well plate may be inspected up to approximately 12 times. If there are thousands of micro-well plates that require inspection, it can be a very time consuming task to manually move the micro-well plate from its storage location, place it under a microscope, record the results, and then move it back to its appropriate storage location. Moreover, there is tremendous opportunity for a technician to forget where a particular micro-well plate belongs. Or, a technician handling such a large quantity of micro-well plates can easily drop or otherwise damage the micro-well plates he is handling.

What is needed is a better device and method for storing and retrieving trays containing micro-well plates.

### SUMMARY OF THE INVENTION

The present invention provides a device and method for the automated storage and retrieval of trays holding subject matter. A plurality of trays is inserted into an access device. A computer system is programmed to control a storage gantry to move the trays between the access device, a storage rack and a work cell gantry. The computer system is also programmed to control the work cell gantry to move the subject matter to and from an automated receiving machine. In a preferred embodiment, the subject matter in the trays is a plurality of micro-well plates in which microscopic crystals may be growing and the automated receiving machine is configured to inspect and classify microscopic crystals. The inspecting and classifying device has an indexing device for sequentially placing microscopic crystals in camera-view of a camera and a control computer is programmed to control the indexing device and to cause the camera to take images of the microscopic crystals and then transfer the images to a

classifying processor where the images are classified. In a preferred embodiment, the microscopic crystals are protein crystals that have been grown in the wells of micro-well plates.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a preferred embodiment of the present invention.

FIG. 2 shows a micro-well plate.

FIG. 3 shows a top view of the fixture plate.

FIGS. 4 and 5 show top views of micro-well plates on the fixture plate.

FIG. 6 shows a block diagram of a preferred embodiment of the present invention.

FIG. 7 shows a preferred monitor.

FIGS. 8 – 10 and 18 – 25 show steps in the sequence of operations of a preferred embodiment of the present invention.

FIG. 11 shows hanging drops of liquid in a micro-well plate.

FIG. 12 shows an example of aqueous drop in oil protein crystallization.

FIG. 13 shows a top view of a micro-well plate on the fixture plate.

FIG. 14 shows a side view of the light source shining upwards onto a micro-well plate.

FIG. 15 shows a magnified view of two wells of a micro-well plate, wherein each well has a drop of liquid.

FIGS. 16 and 17 show a detail view of the drops of liquid shown in FIG. 15.

FIG. 26 shows a preferred monitor screen after a run has been completed.

FIGS. 27 and 28 show details of other preferred monitor screens.

FIG. 29 shows a hanging drop of liquid with crystal growth.

FIG. 30 shows a preferred embodiment of the present invention.

FIG. 31 shows a flowchart of an auto-focus subroutine of the present invention.

FIG. 32. shows a flowchart of a focus value subroutine.

FIG. 33 shows a flowchart of the auto score and classify subroutine.

FIGS. 34a – 34d show flowcharts of the classify subroutine.

FIG. 35a–35b show the sub-classification of the crystal class.

FIG. 36 shows the main program flow.

FIG. 37 illustrates a side view illustrating dual filters in the light path.

FIG. 38 illustrates a top view of the drive mechanism for the rotatable linear polarized filter.

FIG. 39 illustrates a top view of a second filter wheel.

FIG. 40 shows the connectivity of another preferred embodiment.

FIGS. 41A and 41B shows another preferred embodiment of the present invention.

FIG. 42 shows the top view of a preferred tray.

FIG. 43 shows a preferred work cell area.

FIG. 44 shows a block diagram of a preferred embodiment of the present invention.

FIGS. 45 – 77 show a sequence of operation of a preferred embodiment of the present invention.

FIG. 78 shows a perspective view of a preferred tray.

FIG. 79 shows another view of the tray of FIG. 78.

FIG. 80 shows another view of the tray of FIG. 78.

FIG. 81 shows another view of the tray of FIG. 78.

FIG. 82 shows another view of the tray of FIG. 78.

#### DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

A detailed description of a preferred embodiment of the present invention can be described by reference to the drawings.

FIG. 1 shows a preferred embodiment of the present invention. Micro-well plates 125A – 125F are placed on fixture plate 129. In a preferred embodiment, each micro-well plate has 96 wells. Each well has a drop of liquid in which microscopic protein crystals may be growing. Computer 105 automatically controls linear actuators 115, 150 and 160. Linear actuator 115 moves fixture plate 129 along the x-axis. Linear actuator 150 moves moving base 154 along the y-axis, and linear actuator 160 moves moving plate 162 along the z-axis. The computer coordinates the movement of the linear actuators to properly position cameras 155 and 135 above each well of each micro-well plate 125A – 125F in sequence. Preferably, cameras 155 and 135 are high-resolution 2/3 inch CCD cameras, with 1,300 horizontal pixel elements by 1,030 vertical elements. Each element is preferably 6.7 microns square with a sensing area of 8.7 mm by 6.9 mm. Cameras 155 and 135 take images of each well and transmit the images to computer 105 where they are digitized into a image data array of approximately 1,296 horizontal pixels by 1,000 vertical pixels by 8 bits of gray level representing the intensity (0 to 255) of each pixel. These digitized images are automatically recorded in the database of computer 105 and can be analyzed and



scored by an operator via monitor 620. The digitized images may be further processed, analyzed, and the contents of the individual well scored by computer 105 executing program instructions to perform calculations on the image data array. In a preferred embodiment, computer 105 is connected via a communication/control line to a computer network. In this manner, the present invention can be controlled from a remote computer. Likewise, images and data can be transmitted to the remote computer.

### Sequence of Operation of a Preferred Embodiment

#### Micro-well plates loaded onto the System

As shown in FIG. 1, six 96-well micro-well plates 125A – 125F have been placed (either by an operator or by an external loading robot) onto fixture plate 129. A 96 well micro-well plate 125A is shown in FIG. 2. Micro-well plate 125A has wells labeled A1 through H12 and a bar code label 220. Micro-well plate 125A is available from Nalge-Nunc International, with U.S. offices in Rochester, NY. Fixture plate 129 will hold 24, 48, 96, 384, or 1536 well micro-well plates since the micro-well plate external width and length are fairly standard in the industry. The 96 well micro-well plate will be used to illustrate the present invention.

FIG. 3 shows a top view of fixture plate 129 just prior to loading micro-well plates 125A – 125F. Fixture plate 129 has six cutout sections 131 that are just slightly smaller in length and width than micro-well plates 125A – 125F.

FIG. 4 shows a top view of micro-well plates 125A – 125F immediately after they have been placed on fixture plate 129.

After the operator has placed micro-well plates 125A – 125F onto fixture plate 129 as shown in FIG. 4, he enters the command into computer 105 (FIG. 1 and FIG. 6) to expand detents 510 and 520 (FIG. 5). The expansion of detents 510 and 520 firmly secures micro-well plates 125A – 125F against plate stops 530.

### Recording the Bar Code Information for the Micro-well plates

Computer 105 (FIG. 1) has been programmed to accept inputs from an operator. FIG. 7 shows a display representing micro-well plates 125A – 125F on the screen of monitor 620. In FIG. 7, the operator has mouse clicked on bars 622A – 622F, which has caused them to turn green. By clicking on bars 622A – 622F, the operator has selected corresponding micro-well plates 125A – 125F to “run”. The operator sends the command to run the selected micro-well plates by clicking on run bar 623.

In FIG. 8, the operator has given the command to run the selected micro-well plates. Micro-well plate 125A has been moved to a position underneath cameras 155 and 135. By the utilization of plate sensor transmitter/receiver 186 and reflector 188, information is sent to computer 105 reporting that micro-well plate 125A is in position underneath the cameras. Plate sensor transmitter/receiver 186 is fixed to support 189 and is aligned to sense whenever a micro-well plate breaks a beam of light emitted by transmitter/receiver 186 and reflected by a reflector 188. Reflector 188 is mounted on support 191 on the opposite side of the linear actuator 115. The plate sensor transmitter/receiver 186 and reflector 188 are preferably model # E3T-SR13 available from Western Switch Controls of Santa Ana, CA.

Bar code reader 175 is also mounted to support 189 and is positioned to view bar-code identity label 220 (FIG. 2) attached to micro-well plate 125A when it is positioned underneath cameras 155 and 135. Bar-code reader 175 is preferably model # BL601 available from Keyence Corporation of America of Newark, NJ. Bar-code reader 175 communicates with computer 105 via a communication line. Information encoded into label 220 preferably includes: the plate serial number, the plate type (i.e., 24-well, 48-well, 96-well, 384-well, or 1536-well micro-well plate), and the well type (i.e., square, or rounded, hanging drop, sitting drop, constrained sitting drop).

The information from plate sensor transmitter/receiver 186 and bar-code reader 175 is transmitted to computer 105 and stored for later use during the camera inspection and information acquisition phase.

In FIG. 9, linear actuator 115 has moved fixture plate 129 so that micro-well plate 125B is underneath cameras 155 and 135. In a fashion similar to that described above

with regards to micro-well plate 125A, information is transmitted from plate sensor transmitter/receiver 186 and bar-code reader 175 to computer 105 and stored for later use during the camera inspection and information acquisition phase.

The above described sequence continues until all micro-well plates 125A – 125F have been sensed and recorded by plate sensor transmitter/receiver 186 and bar-code reader 175.

Then, as shown in FIG. 10, linear actuator 115 moves micro-well plate 125A so that it is underneath lens 165 of camera 155. Motor 130 of linear actuator 160 moves moving plate 162 upward and/or downward as necessary to properly focus lens 165 on the drop of hanging liquid over well A1. Preferably, lens 165 is set at a predetermined zoom.

### Inspection of Crystals

#### Determining the Position of the Drop of Liquid within Each Well

An operation to inspect each well to determine the position of each hanging drop of liquid is performed on micro-well plate 125A after it has been moved to the position shown in FIG. 10.

FIG. 11 shows a cross section side view of wells A1 – B1 of micro-well plate 125A. In a preferred embodiment, an attempt has been made to grow protein crystals in the hanging drops in each of the wells of micro-well plate 125A. FIG. 11 shows hanging drops  $\alpha 1$ ,  $\beta 1$ ,  $\chi 1$ ,  $\delta 1$ , and  $\epsilon 1$ .

The preferred method for protein crystal growth is the hanging drop method. The hanging drop method (also known as vapor diffusion) is probably the most common method of protein crystal growth. As explained in the background section, a drop of protein solution is suspended over a reservoir containing buffer and precipitant. Water diffuses from the drop to the solution leaving the drop with optimal crystal growth conditions.

In FIG. 10, lens 165 of camera 155 is over well A1 of micro-well plate 125A (FIG. 2, FIG. 11, and FIG. 13). FIG. 13 shows a top view of micro-well plate 125A positioned on fixture plate 129.

FIG. 14 shows a side view of micro-well plate 125A positioned on fixture plate 129. Support 191 with embedded light source 194 is positioned to the side of fixture plate 129. Light from light guide 195 is directed upward through cutout 131 (also shown in FIG. 3). Light guide 195 is positioned between fixture plate 129 and plate 127 such that both plates can move around the light guide 195 without interference. As explained above, fixture plate 129 has cutouts 131 (FIG. 3) that are smaller than the micro-well plates 125 and located under each well plate, such that light from light guide 195 can be projected through the well plates when they are brought into position for inspection. In the preferred embodiment, light source 194 is model # A08925 fiber-optic backlight available from Aegis Electronics of Carlsbad, CA.

Camera 155 (FIG. 10) inspects well A1 and transmits an image to computer 105 for digitization. As described above, camera 155 preferably (FIG. 10) inspects well A1 at a 1X magnification so that every 6.7 micron square pixel represents approximately 6.7 square microns on the object being measured, allowing for some small geometric distortions caused by the lens 165. Computer 105 has been programmed to digitize the camera image and then by utilizing vision software determines a position within well A1 for the drop of liquid hanging from grease seal 361. The position of the drop of liquid is recorded for later use onto the hard drive of computer 105 and to a memory location within the computer.

In a preferred embodiment, the vision software used to determine the position of the drop of liquid uses a software routine algorithm called `mvt_blob_find` from a collection of image processing tools called MVTools. MVTools is available from Coreco Imaging, US Office in Bedford, MA.

After recording the position of the drop of liquid hanging from grease seal 361 in well A1, linear actuator 115 moves fixture plate slightly to the left so that lens 165 is over well B1 (FIG. 13). In a fashion similar to that described for well A1, the position of the drop of liquid hanging from grease seal 361 in well B1 is recorded on the hard

drive of computer 105 and in computer memory. For example, as shown in FIG. 15, computer 105 will record that drop of liquid  $\alpha 1$  is towards the upper left-hand quadrant of well A1. Likewise, the position of drop of liquid  $\beta 1$  is recorded onto the database of computer 105 as being in the lower right-hand quadrant of well B1.

In this manner, positions of the drops of liquid are recorded for cells A1 – H12. In FIG. 18, linear actuator 115 has moved fixture plate 129 so that well H1 is under lens 165.

In FIG. 19, linear actuator 115 has moved fixture plate 129 to the left and linear actuator 150 has moved moving base 154 slightly rearward so that lens 165 is over well A2 of micro-well plate 125A (FIG. 13).

In a manner similar to that described above, positions of the drops of liquid are recorded for cells A2 – H12 (FIG. 2, FIG. 13). In FIG. 20, linear actuator 115 has moved fixture plate 129 to the left and linear actuator 150 has moved moving base 154 rearward so that well H12 is under lens 165.

After positions of the drops of liquid are recorded for cells A1 – H12 for micro-well plate 125A, linear actuator 115 moves fixture plate 129 and linear actuator 150 moves moving base 154 so that cell A1 of micro-well plate 125B is underneath lens 165 (FIG. 21).

In a manner similar to that described above, positions of the drops of liquid are recorded for cells A1 – H12 for each micro-well plate 125A – 125F. In FIG. 22, linear actuator 115 has moved fixture plate 129 and linear actuator 150 has moved moving base 154 so that well H12 of micro-well plate 125F is under lens 165.

#### Recording the Image of the Drop of Liquid within Each Well

An operation to inspect each hanging drop is performed at a higher magnification using camera 135 with its zoom lens 145 capable of magnifications of 2.5X to 10X corresponding approximately to digitized pixels representing 2.68 microns square (at 2.5X) to 0.67 microns square (at 10X). This inspection is done for the purpose of

determining whether protein crystals have grown. Zoom motor 192 controls the degree of zoom for zoom lens 145. Using data representing the position of the drop of liquid within each well obtained during the inspect-well sequence, computer 105 (FIG. 1) automatically transmits a signal to linear actuators 115 and 150 to position lens 145 directly over the drop of liquid within each well. For example, in FIG. 23 lens 145 is positioned over the top of well A1 of micro-well plate 125A. Using the positioning data earlier obtained, lens 145 is precisely positioned so that it is able to zoom in on drop of liquid  $\alpha$ 1 (FIG. 13). FIG. 16 shows a magnified view of drop of liquid  $\alpha$ 1. In FIG. 23, motor 130 of linear actuator 160 has moved moving plate 162 upward and/or downward as necessary to properly focus lens 165 on drop of liquid  $\alpha$ 1. Zoom motor 192 has manipulated lens 165 to obtain the desired degree of zoom. Camera 135 inspects well A1 and transmits a signal representing the magnified image of the hanging drop of liquid to computer 105. The images are stored on computer 105 temporarily in memory for immediate analysis and on hard disk for later analysis.

In a similar fashion, linear actuators 115, 150 and 160 and zoom motor 192 operator to properly position and magnify zoom lens 145 over each hanging drop of liquid to obtain desired focus and magnification for image data storage. For example, FIG. 17 shows a magnified view of hanging drop of liquid  $\beta$ 1.

In a manner similar to that described above during the inspect-well sequence, magnified images of the drops of liquids (similar to those shown in FIGS. 16 and 17) are recorded for cells A1 – H12 for micro-well plate 125A (FIG. 2, FIG. 13). In FIG. 24, linear actuator 115 has moved fixture plate 129 and linear actuator 150 has moved moving base 154 so that well H12 of micro-well plate 125A is under lens 165.

Then, the sequence is repeated for micro-well plates 125B – 125F so that magnified images of the hanging drops of liquids are recorded for each cell A1 – H12 for micro-well plates 125B – 125F. In FIG. 25, the sequence has ended for micro-well plates 125A – 125F. Linear actuator 115 has moved fixture plate 129 and linear actuator 150 has moved moving base 154 so that well H12 of micro-well plate 125F is under lens 145.

### Manual Scoring the Drop of Liquid within Each Well

After micro-well plates 125A – 125F have been run, monitor 620 will appear as shown in FIG. 26. In FIG. 26, six images representing micro-well plates 125A – 125F appear on the screen. Above each image is a message “Run Comp” indicating that image data for hanging drops of liquid has been transferred into computer 105. Beneath each image are buttons 710 – 715 marked “S”. By mouse clicking on any button 710 – 715, the operator may manually score for successful crystal formation each magnified image of each hanging drop of liquid.

For example, in FIG. 26, the operator can mouse click on button 710 to score micro-well plate 125A.

In FIG. 27, the operator has mouse clicked on the circle representing well A1 of micro-well plate 125A. This has caused a magnified image to be displayed of drop of liquid  $\alpha 1$  in screen section 716. The operator has concluded that there are no crystals in drop of liquid  $\alpha 1$  and has therefore mouse clicked on button 717 for “NO CRYSTAL”. On the display screen, this has caused the circle representing well A1 of micro-well plate 125A to turn red.

In FIG. 28, the operator has mouse clicked on the circle representing well B1 of micro-well plate 125A. This has caused a magnified image to be displayed of drop of liquid  $\beta 1$  in screen section 716. The operator has concluded that there are crystals in drop of liquid  $\beta 1$  and has therefore mouse clicked on button 718 for “CRYSTAL”. On the display screen, this has caused the circle representing well B1 of micro-well plate 125A to turn green.

In a similar fashion, the above scoring procedure is repeated until all wells A1 – H12 for micro-well plates 125A – 125F have been scored as either red (NO CRYSTAL) or green (CRYSTAL).

### Data Utilization

Once micro-well plates 125A – 125F have all been scored, the operator has at his easy disposal a database that contains the identity of each micro-well plate that was

inspected along with a score summarizing whether crystal formation occurred for each well in the micro-well plate. The automated and efficient manner in which the operator is able to acquire his contrasts with the prior art method of laboriously inspecting each well with a microscope and the handwriting the results into a notebook.

For example, to score six 96-well micro-well plates utilizing the present invention should take approximately no more than 10 to 15 minutes.

In contrast, the prior art method of inspecting six 96-well micro-well plates with a microscope and the handwriting the results into a notebook will take approximately 30 to 100 minutes depending on the conditions discussed in the background section in addition to the time required to transcribe the results into a computer database. Plus, as previously explained in the background section, manual inspection and scoring is subject to a relatively high risk of human error.

#### Second Preferred Embodiment

In a second preferred embodiment, the depth of view of camera 135 is approximately 50 to 100 micrometers. The crystal in the drop of liquid may be larger than the depth of view or there may be crystals growing at various levels within the hanging drop of liquid, as shown in FIG. 29. Therefore, in the second preferred embodiment, lens 145 is focused at multiple different levels 721 - 724 and a set of images are recorded at the different levels so that the entire crystal may be analyzed.

#### Specimen Auto-Focus

The third preferred embodiment of the present invention utilizes a specimen auto-focus subroutine 300 (FIG. 31). Subroutine 300 ensures that the specimen within the micro-well is in-focus at the desired zoom (or magnification ratio) of image lens 145. Utilizing the auto-focus feature, the present invention causes camera 135 to take a number of images defined by a Number\_of\_Z\_Slices. Typically, there are between 5 and 10 slices separated in the Z-axis from one another by a Z\_Step\_Size. The typical step size is 0.05mm to 0.25 mm. The slices preferably start at a Z-Axis location defined by a Start\_Z value, which is typically at the bottom of the cover-slip on the micro-well plate. During specimen auto focus initialization 302, input data 304 is



received. The area of interest window within the images is further defined in the input data 304 by a `X_Window_Center` and a `Y_Window_Center` plus an `X_Width` and a `Y_Width`. Initial settings 306 for the routine 300 are the starting value of a counter `N`, a `Best_Focus`, a `Best_Z`, and a `Focus_Error`. The inspection device sets `Z_Position(N)` equal to the `Start_Z` location and moves camera 135 there in step 308. An image is acquired with camera 135 and digitized as previously described and stored as `Image(N)` in step 310. A second subroutine 312 extracts a focus value `F(N)` for `Image(N)` and is further described in the section for discussion for FIG. 32. A test is made between `F(N)` and the `Best_Focus` in step 316, such that if `F(N)` is greater than `Best_Focus` then `Best_Focus` is set to `F(N)` and `Best_Z` is set to `N` as shown in step 320 and the program flow goes onto step 322, if the test condition is not met in step 316 then the program flow skips step 320 and goes on to step 322. In step 322, a test is made to determine if all of the slices have been taken as `N` is tested against the `Number_of_Z_Slices`. If `N` is equal to `Number_of_Z_Slices` then program flow goes onto step 324. If more slice images are needed, then the flow goes to step 318. In step 318, `Z_Position(N+1)` is set to `Z_Position(N) + Z_Step_Size` and the Z-Axis is moved to `Z_Position(N+1)` and the program flow goes on to step 314 where `N` is incremented by 1 (one). The program flow goes back to step 310 and completes the loop of step 310 to step 322 until all of the image slices have been taken and then moves onto step 324. In step 324, `Best_Z` is tested against its initial value, and if it equals its initial value (meaning no focus was found in the focus value subroutine 312) then it is set to a default value of the `Number_of_Z_Slices` divided by 2 and `Focus_Error` is set to 1 (one) in step 326 and the program flow goes onto step 328. If `Best_Z` in step 324 has a value other than its initial value then program flow goes onto step 328 from step 324. In step 328 a `Best_Image` image is set to the image slice at best focus by setting `Best_Image` equal to `Image(Best_Z)`. Also, a `Best_Image_Z` value is set equal to `Z_Position(Best_Z)` and the flow goes onto step 330 which is the RETURN part of the subroutine and program flow returns to the main software flow.

As illustrated in FIG 32, an `image(N)` focus `F(N)` subroutine 312 is further detailed, starting at the step `Start` 401. A `pointer_to_image(N)` 404 is provided in step 402. In step 408 the `image(N)` is convolved with a standard 3 x 3 Sobel\_Horizontal filter 416 to produce an `Image(N)_H` wherein horizontal edges within the image are emphasized. In step 410 the `image(N)` is convolved with a standard 3 x 3

Sobel\_Vertical filter 418 to produce an Image(N)\_V wherein vertical edges within the image are emphasized. In step 414, both the horizontal edge emphasized image Image(N)\_H and the vertical edge emphasized image Image(N)\_V are summed pixel by pixel, during the summing process any resulting negative pixel values are set to zero and any resulting pixel values that are greater than 255 are set to 255, to produce an image Image(N)\_Focus. In step 420, a simple variance F(N) is calculated for the pixels within a window of interest defined in 422 by X\_Window\_Center, Y\_Window\_Center, X\_width, and Y\_Height. The resulting value of the variance is returned to the calling program as F(N) in step 424. The sobel processing and the variance calculation is performed with a collection of image processing software tools within MVTools. MVTools is available from Coreco Imaging, US Office in Bedford, MA.

#### Fourth Preferred Embodiment

In the first preferred embodiment, it was disclosed how an operator could manually score each drop of liquid as either "CRYSTAL" or "NO CRYSTAL". In the fourth preferred embodiment, the operator is given a greater variety of options in deciding on how to score each drop. Table 1 shows listing of the operator's scoring options, including number, text description, and the corresponding color code. Once a micro-well drop has been scored a 9, the operator can further classify the crystals in a scoring shown in Table 2.

TABLE 1:

SCORE	DESCRIPTION	DISPLAY COLOR
0	clear	White
1	light precipitation	Red
2	heavy precipitation	Yellow
3	ugly precipitation	Blue
4	phase separation	Orange
5	unknown	Violet
6	Spherulites	Black
7	Grainy precipitation	Gray
8	Microcrystals	Brown
9	Crystal	Green

TABLE 2:

SCORE	DESCRIPTION
9.0	crystal (no comments)
9.1	needles, intergrown
9.2	needles, single
9.3	plates, intergrown
9.4	plates, single
9.5	chunks, < 50microns, intergrown
9.6	chunks, < 50 microns, single
9.7	chunks, > 50 microns, intergrown
9.8	chunks, > 50 microns, single
9.9	gorgeous > 50 microns

#### Fifth Preferred Embodiment

In the fourth preferred embodiment, it was disclosed how an operator can manually score each drop of liquid into one of 10 categories with corresponding color coding, and how the operator can score category 9 into further subcategories of 9.0 through 9.9. In the fifth preferred embodiment, the inspection device automatically scores and classifies each drop specimen by executing computer software subroutines as shown in FIGS. 33, 34a, 34b, 34c, 34d, and 35a and 35b under control of the program flow shown in FIG 36. The automatic classification can occur at three levels of detail, the first level, Type\_of\_Classification = 1, simply discriminates between a drop that is clear or not-clear (unknown), the second level, Type\_of\_Classification = 2, scores and

classifies the drop into classes 0 through 9 as described in Table 1 above, and the third level, `Type_of_Classification = 3`, performs second level scoring and classification, plus adds an additional 10 subcategories to the CLASS 9, crystal classification, as detailed in Table 2 above.

#### Automatic Scoring and Classification

FIG. 36 illustrates the main program flow 840 starting at step 842. The software is initialized with parameters, inspection lists, and `Type_of_Classification` detailed in step 486. The flow continues onto step 844 where the system moves the micro-well of interest within the selected micro-well plate under the selected camera. In step 851, if the drop needs to be located, the flow continues onto step 849 wherein an image is acquired by the camera and software operates on the image and determines the location of the drop. Then a test is made to determine if the last micro-well in the plate has been imaged, if not then the flow loops to step 844 and continues. If the last micro-well in plate step 853 has been imaged then the flow continues to step 856 where the system moves to the droplet within micro-well in micro-well plate under the high-resolution camera. Also in step 851 if the drop had been previously located, then the flow would continue from step 844 directly to step 856 without the need to re-locate the drop. From step 856, the flow continues onto step 857 wherein a high-resolution image of the drop is obtained. Then the flow goes onto step 850. In step 850, a CALL is made to subroutine that automatically scores and classifies the drop depending on the `Type_of_Classification` required. The subroutine is detailed in FIG. 33 and starts at step 440 in FIG. 33. After the drop has been classified the subroutine returns to step 852 wherein the results are stored and reported. The program flow continues to step 858 where a test is made to determine if the last drop in the selected plate has been processed, if so then the flow goes onto step 848 wherein a test is made to determine if the last plate has been processed. If not, the flow loops back to 856 and continues. If the last plate has been processed, the flow goes onto step 854 and the program is done.

FIG. 33 shows Micro-well Specimen Auto Score and Classify Subroutine 438 starting at step 440. `Pointer_to_Best_Image` 442 provides information to the initialization step 444 that allows access to the image that was found to be the best focus. Plus, the `Type_of_Classification` is passed into the routine. Alternatively, pointer 442 can point

to an image that was taken at a z height value known to be the focus of the system. After initialization 444 the subroutine 438 calculates in step 448 an average\_gray\_Background value normalized to allow for the variation present from various plate types, by using a first rectangular window defined by parameters shown in step 446 (X\_Background\_Center, Y\_Background\_Center, X\_Background\_Width, and Y\_Background\_Height) and by summing all of the gray scale values of the pixels defined by the window 446 and dividing by the number of pixels within that window. The average\_gray\_background is normalized for well-type differences by multiplying the calculated value by a Micro-well normer value also found in step 446 and generally determined by measuring the various micro-well plate types under inspection and normalizing to the 96-well standard micro-well plate. This average\_gray\_background 448 is calculated in a window area of the image that is outside the area of the drop but generally within the well or within the bounding well walls.

In step 450 an average\_gray\_Classify\_Window value is calculated in a similar manner as described above (except it is not normalized for micro-well type) using a second rectangular window defined by parameters shown in step 452 (i.e., X\_Classify\_Center, Y\_Classify\_Center, X\_Classify\_Width, and Y\_Classify\_Height). This average\_gray\_classify\_window value 450 is taken in a rectangular window area of the image that is inside the area of the drop and defined by being a fraction between 0.98 and 0.5 (with 0.8 preferred) of the width and height of the external bounding rectangular box from the blob utilizing subroutine mvt\_blob\_find. The subroutine mvt\_blob\_find defines the extent of the drop as previously discussed in the section "Determining the Position of the Drop of Liquid within Each Well".

In step 454, a diagonal difference image is calculated by stepwise subtracting pixel values from two pixel locations, defined by (x,y) and (x+Diff\_Sep, y+Diff\_Sep) within the Classify\_window. The pixel values are separated in width and height by the value Diff\_sep from step 452. This is repeated over all pixels within the Classify window defined by step 452 using X\_Classify\_Center, Y\_Classify\_Center, X\_Classify\_Width, and Y\_Classify\_Height. For each value calculated the absolute value is taken of the subtraction result and compared to a threshold value Flag\_Thresh defined in step 452. If the calculated value is greather than Flag\_Thresh 452 then the

pixel is set at the first location in x,y equal to a value defined by Set1 in step 452, if the calculated value is equal to or less than Flag\_Thresh, 452, then the pixel value is set to zero. This can be seen by the mathematical equations and flow described in step 454 in calculating a Diff\_Image\_1. Typical values for Diff\_Sep are between 1 to 20 pixels with 7 preferred. Typical values for Set1 are between 1 and 511 with 128 preferred. Typical values for Flag\_Thresh are between 5 and 50 with 25 preferred.

In Step 456, a calculation similar to that performed in step 454 is performed on the Classify\_Window except that the separation between the two pixels undergoing the calculation is defined by (x+Diff\_Sep, y) and (x, y+Diff\_Sep), as is shown in the mathematical calculation in 456 to generate a Diff\_Image\_2. This calculation uses definitions shown in step 452. Typical values for Set2 are between 1 and 511 with 200 preferred.

In Step 458 the Classify\_Image, which is a combination of the images generated in step 454 and 456 is calculated as shown by the mathematical equations shown in step 458 using definitions shown in step 452. If the x,y pixel value in either Diff\_Image\_1 or Diff\_Image\_2 (steps 454 and 456 respectively) has a value equal to Set1 452 then the pixel value is set at (x,y) in Classify\_Image equal to Set2 452. Otherwise, the value is equal to zero(0) as shown in the mathematical equations in step 458. The calculations are repeated for all pixels within the window defined in 452. The Classify\_Image is basically an image of the classify\_image\_window wherein edges present within the original Best\_Image are detected.

In step 460, the value of number\_Pixels\_Set2 is set equal to the total number of pixels that are set equal to Set2 452 in step 458. Also, the value of Total\_Pixels\_in\_Window is set to the total number of pixels in the Classify\_window in step 458.

In step 462, a Score\_Gray is calculated by dividing the Average\_Gray\_Classify\_Window determined in step 450 by the Average\_Gray\_Background found in step 448. A Score\_Flag is also calculated by dividing the Number\_Pixels\_Set2 by Total\_Pixels\_in\_Window from step 460. The Score\_Gray and Score\_Flag are normalized in this matter.

In step 464, the values of Score\_Gray, Score\_Flag, and Type\_of\_Classification are passed to a classify subroutine and a classification is returned for the Classify\_image, effectively classifying the protein crystals within the window. Details of the Classify subroutine 464 are provided in FIG 34a, 34b, 34c, and 34d, plus FIG 35a and 35b.

FIG 34a shows the Classify Subroutine 464. Start of Classify subroutine is shown in step 468 followed by initialization 470 whereby the initial classification CLASS value is set to 5, representing "unknown" and the flow goes onto step 480.

Step 480 calculates whether the drop is clear and the CLASS = 0 by a test detailed in step 480 using thresholds defined in step 482 (Clear\_Flag\_LT and Clear\_Gray\_GT) with the following equation: if score\_flag is less than Clear\_Flag\_LT and Score\_Gray is greater than Clear\_Gray\_GT then set CLASS = 0. At step 481, a test is made to see if the type\_of\_classification is equal to 1, the first classification type wherein the drop is classified as simply clear (0) or unknown (5) as previously discussed. If the type\_of\_classification is equal to 1 then the flow goes on to step 483 and then returns to FIG. 33 step 466 with the results. If the type\_of\_classification is not equal to 1 then the flow goes onto step 476 for further classification.

Step 476, utilizing threshold value parameters shown in step 478 (Lgt\_Precip\_Flag\_LT, Lgt\_Precip\_Flag\_GT, Lgt\_Precip\_Gray\_GT), assigns the value of 1 to CLASS indicating that Light Precipitation is present in the Classify\_Image. Step 476 utilizes the mathematical equation which states if Score\_Flag is less than Lgt\_Precip\_Flag\_LT and Score\_Flag is greater than Lgt\_Precip\_Flag\_GT and Score\_Gray is greater than Lgt\_Precip\_Gray\_GT then set CLASS to value 1.

Step 484 calculates heavy precipitation by using thresholds detailed in step 486 (Heavy\_Precip\_Flag\_LT, Heavy\_Precip\_Flag\_GT, Heavy\_Precip\_Gray\_LT) with the following equation: if score\_flag is less than Heavy\_Precip\_Flag\_LT and score\_flag is greater than Heavy\_Precip\_Flag\_GT and score\_gray is less than Heavy\_Precip\_Gray\_LT then set CLASS = 2.

Step 488 calculates ugly precipitation by using thresholds detailed in step 490, Ugly\_Precip\_Flag\_LT, Ugly\_Precip\_Flag\_GT, Ugly\_Precip\_Gray\_LT, with the following equation: if score\_flag is less than Ugly\_Precip\_Flag\_LT and score\_flag is greater than Ugly\_Precip\_Flag\_GT and score\_gray is less than Ugly\_Precip\_Gray\_LT then set CLASS = 3.

Step 492 continues the classification process in FIG 34b.

In FIG 34b, the continuation of the classification process 700 is shown continuing in step 702. Step 704 calculates micro\_crystals by using thresholds detailed in step 703 (Micro\_Cry\_Flag\_GT and Micro\_Cry\_Gray\_LT) with the following equation: if score\_flag is greater than Micro\_Cry\_Flag\_GT and score\_gray is less than Micro\_Cry\_Gray\_LT, then set CLASS = 8.

Step 704 calculates crystals by using thresholds detailed in step 705 (Crystal\_Flag\_LT, Crystal\_Flag\_GT, and Crystal\_Gray\_GT) with the following equation: if score\_flag is less than Crystal\_Flag\_LT and score\_flag is greater than Crystal\_Flag\_GT and score\_gray is greater than Crystal\_Gray\_GT then set CLASS = 9.

Step 706 calculates Grainy precipitation by using thresholds detailed in step 707 (Grainy\_Flag\_GT, and Grainy\_Gray\_GT) with the following equation: if CLASS = 8 and score\_flag is greater than Grainy\_Flag\_GT and score\_gray is greater than Grainy\_Gray\_GT, then set CLASS = 7.

Step 708 continues the classification process onto FIG 34c as 725.

Step 726 continues from step 708 of FIG 34b.

In FIG 34c, Step 727 calculates and generates a first set of additional image features for further use in classification taking as input 726 (X\_Classify\_Center, Y\_Classify\_Center, X\_Classify\_Width, Y\_Classify\_Height, Pointer\_to\_classify\_image, and mvt\_blob\_analysis\_Params). In step 727, MVT\_Tools\_Blob\_Analysis with pointer to window in Classify\_Image is called. Step 727 gets the num\_found of blobs and, for



each blob found, step 727 gets its area(m), height(m), width(m), perimeter(m), and location of each as location\_X(m) and location\_Y(m). These values are recorded. These calculations are performed on the image called Classify\_Image, which is the image that was formed in FIG 33 as step 458.

In step 728, if num\_found is not greater than zero(0) the subroutine goes to step 738. However, if any blobs are found then further analysis is started in step 729 by setting m=1, num\_spherolite = 0, and spherolite(m) = 0. In step 730, the following blob\_ratios are calculated:  $\text{Circle\_Like\_HW}(m) = \text{height}(m)/\text{Width}(m)$  and  $\text{Circle\_Like\_AHW}(m) = \text{area}(m)/(\text{height}(m)*\text{width}(m))$ . For blobs that are circular, Circle\_Like\_HW will be around a value of one (1). If blobs get elongated then the value will be other than one. Circle\_Like\_AHW for circular blobs has a value around 0.785. For square-like blobs the value will be closer to one (1). The program flow goes onto step 732.

Step 732 determines whether to classify a drop as having a spherolite by utilizing the parameters found in 734 (Circle\_Like\_HW\_lower, Circle\_Like\_HW\_Upper, Circle\_Like\_AHW\_Lower, and Circle\_Like\_AHW\_Upper). The following equation is used:: if CLASS = 8 or 9 and Circle\_Like\_HW(m) is less than Circle\_Like\_HW\_Upper and Circle\_Like\_HW(m) is greater than Circle\_Like\_HW\_lower, and Circle\_Like\_AHW(m) is less than Circle\_Like\_AHW\_Upper and Circle\_Like\_AHW(m) is greater then Circle\_Like\_AHW\_Lower, then num\_spherolite = num\_spherolite +1. An increment is done by calculating m = m+1 and setting spherolite(m) = 1 to show one has been found at m.

Step 736 tests whether all of the found blobs have been classified by testing m against num\_found and, if equal, the subroutine goes onto step 738. If not the program loops back to step 730 and flows through as above.

Step 738 goes onto FIG 34d in the classification subroutine as 740.

FIG 34d continues from FIG 34c at 742 and goes to 744 where a second set of additional features is calculated. These features are generated with the original

Best\_Image generated in step 328 (FIG. 31) as the image of the best focus. For these additional features, the results are used from the blob analysis performed in FIG. 34c on the classify\_image. Such features as shown in parameters 746 as num\_found, area(m), height(m), width(m), perimeter(m), location\_X(m), location\_Y(m), num\_spherolite, and spherolite(m). Plus it uses the average\_gray background from FIG. 33 step 448, and the pointer\_to\_Best\_Image in FIG. 31 step 328. In step 744 "m" and num\_phase\_sep are set equal to zero.

In step 748, if the number of spherolites previously found (num\_spherolite) is not greater than zero (0), then the flow goes to step 756 and the remaining steps shown in FIG 34d are bypassed. But if the num\_spherolite is greater than zero (0), then the flow goes onto step 749 wherein m is incremented by 1. Then the flow goes on to step 751 to test whether to go to step 756 or to step 753 depending on comparing "m" to the num\_found. In step 753, the value of spherolite(m) found in FIG. 34c step 732 is tested to see if any were classified as spherolite. If not, then the flow loops back to step 749. If spherolite(m) is equal to one (1) then step 750 is executed.

In step 750, the information location\_X(m), location\_Y(m), one half of height(m) and one half of width(m) is used to calculate the average gray value within this reduced image inside each spherolite. The Best\_Image pixel data obtained from the previous blob analysis is utilized and phase\_sep\_Gray(m) is set equal to this value. Phase\_sep\_Gray(m) is then normalized by dividing it by the Average\_Gray\_Background. The program flow goes onto step 752, wherein phase\_sep\_Gray(m) is tested to see if it is greater than a parameter phase\_sep\_Gray\_GT from parameter input step 754. If true, then CLASS is set equal to four(4) and the program loops up to step 749.

In step 751 the automatic program has classified the microdrop into one of the 10 primary classes, 0 through 9, detailed previously in Table 1. Then, a test is made to see if the type\_of\_classification is equal to 2. If so, then the flow goes onto step 755 wherein the flow is returned to FIG. 33. In step 466, a general classification is complete. If further classification into subcategories is required, then the flow goes onto step 758.

In step 758, the program flow goes onto FIG. 35a step 760.

In FIG. 35a, a crystal classification subroutine 762 further classifies any CLASS 9 crystal image into to additional subclasses, 9.2, 9.4, 9.6, 9.8, or 9.9, as previously discussed in Table 2. The subroutine begins at step 760 and goes onto step 764. In step 764, the blob counter *m* is set to zero. A test is conducted to see if the CLASS is equal to 9 and, if not, the flow goes to step 778 and returns to FIG. 33 step 466. Step 764 uses input values as shown in step 766 (Average\_Gray\_Background, Pointer\_To\_Best\_Image, num\_found, area(*m*), height(*m*), width(*m*), perimeter(*m*), location\_X(*m*), location\_Y(*m*), num\_spherolite, and spherolite(*m*)), as previously described. If the CLASS is equal to 9 another test is made to see if one or more than one blobs were previously found. If only one blob was previously found then the flow goes onto step 768. If more than one is found, the flow goes onto step 769 in FIG 35b. In step 768, a height to width ratio is calculated and compared to thresholds representing needle-like characteristics in step 770. If the test conditions are met, then the CLASS is set to 9.2. If not, the flow goes onto step 771. In step 771, the height to width ratio is compared to thresholds representing plate-like characteristics in step 772. If the conditions of the test are met, the CLASS is changed to 9.4 and the flow goes onto step 773. If not, no further classification is performed and the flow goes onto step 778 and the subroutine returns to FIG. 33 step 466. In step 773, a normalized average gray value, Chunk\_Gray(*m*), within the blob is calculated and the flow goes onto step 774. In step 774, if Chunk\_gray(*m*) is less than a threshold Chunk\_Gray\_LT (from step 775), then CLASS is set to 9.6 and the flow goes onto step 776. In step 776, if Chunk\_gray(*m*) is greater than a threshold Chunk\_50\_GT, (from step 777), then CLASS is set to 9.8 and the flow goes onto step 780. In step 780 if area(*m*) is greater than threshold gorgeous\_GT (from step 779), then CLASS = 9.9 and the flow goes onto step 778. Then the subroutine returns to FIG. 33 step 466.

FIG 35b shows flowchart 781 illustrating the further classification of a CLASS 9 image having multiple blobs within the image into additional subclasses, 9.1, 9.3, 9.5, or 9.7, as previously discussed in Table 2. The subroutine begins at step 786 from step 769 in FIG. 35a and goes onto step 782. In step 782, a loop begins at *m* equals 0 for each blob *m* using input values from step 784 (Average\_Gray\_Background, Pointer\_To\_Best\_Image, num\_found, area(*m*), height(*m*), width(*m*), perimeter(*m*),

location\_X(m), location\_Y(m), num\_spherolite, and spherolite(m)) as previously described. The flow goes onto step 788 where m is incremented and a height to width ratio is calculated and compared to thresholds representing needle-like characteristics in step 790. If the test conditions are met, then the CLASS is set to 9.1. If not, the flow goes onto step 794. In step 794, the height to width ratio is compared to thresholds representing plate-like characteristics in step 796. If the conditions of the test are met, the CLASS is changed to 9.3. If not, no further classification is performed and the flow goes onto step 812 to determine if all of the blobs have been tested. In step 798, a normalized average gray value (Chunk\_Gray(m) as described before) within the blob is calculated and the flow goes onto step 802. In step 802, if Chunk\_gray(m) is less than a threshold Chunk\_Gray\_LT (from step 804) then CLASS is set to 9.5 and the flow goes onto step 806. In step 806, if Chunk\_gray(m) is greater than a threshold Chunk\_50\_GT (from step 808) then CLASS is set to 9.7 and the flow goes onto step 812. In step 812, if all of the blobs have been tested, the flow goes onto step 810 and the subroutine returns to FIG. 33 step 466. If not all tested, then the flow loops back to step 788 for the next blob and the process loops until complete.

Typical parameter and threshold values for use in classification in FIGS. 33, 34a, 34b, 34c, 34d, and 35a and 35b are given in Table 3 along with the preferred value. These values serve only as a guide, and other values may be used when circumstances justify. For example, different lighting conditions, variations in the transparency of micro-well plates, variations in the formulations of the protein growing media and drop, values may be used as well. One skilled in the art may adjust the parameter and threshold values to tune in the classification results specific to their setup.

Table 3 Typical and preferred values for threshold and Classification parameters

Step	Name	lower value	upper value	Preferred value
452	Diff_Sep	1	20	7
452	Set1	1	511	128
452	Set2	1	511	200
452	Flag_Thresh	5	50	25

478	Lgt_Precip_Flag_LT	0.0001	0.010	0.002
478	Lgt_Precip_Flag_GT	0.0	0.001	0.00001
478	Lgt_Precip_Gray_GT	0.8	1.2	1.020
482	Clear_Flag_LT	0.000001	0.01	0.0007
482	Clear_Gray_GT	0.8	1.2	1.020
486	Heavy_Precip_Flag_LT	0.0001	0.050	0.020
486	Heavy_Precip_Flag_GT	0.0	0.005	0.001
486	Heavy_Precip_Gray_LT	0.7	1.2	0.95
490	Ugly_Precip_Flag_LT	0.0001	0.050	0.020
490	Ugly_Precip_Flag_GT	0.0	0.005	0.001
490	Ugly_Precip_Gray_LT	0.5	1.2	0.7
703	Micro_Cry_Flag_GT	0.0001	0.05	0.020
703	Micro_Cry_Gray_LT	0.9	1.20	1.010
705	Crystal_flag_LT	0.1	0.5	0.30
705	Crystal_Flag_GT	0.001	0.05	0.01
705	Crystal_Gray_GT	0.8	1.2	1.0099
707	Grainy_Flag_GT	.001	.5	0.177
707	Grainy_Gray_GT	0.8	1.2	0.980
734	Circle_Like_HW_lower	0.5	1.00	0.9
734	Circle_Like_HW_Upper	1.00	2.0	1.1
734	Circle_Like_AHW_lower	0.1	3.9	0.78
734	Circle_Like_AHW_Upper	.785	2.0	0.83
754	phase_sep_Gray_GT	0.9	1.2	1.05
770, 790	needle_HW_GT	2	20	5
770, 790	needle_HW_LT	0.5	0.05	0.2
772, 796	plate_HW_GT	1.1	2.0	0.95
772, 796	plate_HW_LT	0.9	1.1	1.05
775, 804	Chunk_Gray_LT	0.8	1.2	1.01
777, 808	Chunk_50_GT	70	200	100
779	Gorgeous_GT	7000	2000	1000

### Prototype

Applicants have designed, built and tested a working prototype of the present invention.

FIG. 30 shows the major components of the Applicant's prototype. Proteomic crystal verification and inspection system 100 has three axis of linear motion. Linear actuator 115 is preferably linear actuator model # 802-0763D available from Dynamic Solutions of Irvine CA., with 600 mm of travel driven by an enclosed 0.5 mm pitch ballscrew. Linear actuator 115 is driven by an intelligent self-contained servo motor 110 model # SM2320 SQ available from Animatics Corp. of Santa Clara, CA., with 38 oz-in of available torque. Servo motor 110 communicates with a Windows Based computer 105 through a serial connection routed through a central control unit 190.

Linear actuator 115 has stationary part 116 fixed to a granite table top 170. Motor 110 moves moving part 117 along the x-axis. Granite top 170 is supported by a frame 180. Frame 180 has casters and adjustable legs. Plate 127 is attached to moving part 117. At each end of plate 127, a spacing block 128 spaces fixture plate 129 from plate 127. At each of its ends, fixture plate 129 is supported by spacing block 128. Fixture plate 129 provides for the mounting, removal, and positioning of multiple micro-well plates 125A – 125F. Preferably micro-well plates 125A – 125F are agar plates, micro-titer well plates of 96, 384, 1536 wells, or other sample plates.

Support 191 is positioned adjacent to fixture plate 129 and contains a light source. In the preferred embodiment, the light source is model # A08925 fiber-optic back light available from Aegis Electronics of Carlsbad, CA.

In the preferred embodiment, linear actuator 150 is model # 802-1384A available from Dynamic Solutions of Irvine CA., with 350 mm of travel driven by an enclosed 0.5 mm pitch ballscrew. Linear actuator 150 has stationary part 153 horizontally bridged above linear actuator 115 and supported by pillars 152. Moving base 154 provides a mounting base for components of linear actuator 160. In a preferred embodiment, linear actuator 150 is driven by an intelligent self-contained servo motor 120 identical to motor 110. Servo motor 120 communicates with

Windows Based computer 105 through a serial connection routed through central control unit 190.

In a preferred embodiment, linear actuator 160 is model # 802-0756D available from Dynamic Solutions of Irvine CA., with 100 mm of travel driven by an enclosed 0.5 mm pitch ballscrew. Linear actuator 160 has with a stationary part 161 mounted perpendicular to and fixed to moving base 154 of linear actuator 150. Linear actuator 160 is driven by intelligent self-contained servo motor 130. Servo motor 130 is preferably identical to motor 110. Servo motor 130 communicates with the Windows Based computer 105 through a serial connection routed through the central control unit 190.

Moving plate 162 provides a mounting base for camera 155. In a preferred embodiment camera 155 is a high-resolution monochrome megapixel CCD camera model # CVM1 from JAI America INC. of Laguna Hills, CA. Camera 155 has lens 165. Preferably, lens 165 has a 0.75x to 3x zoom capability and is model # NT52-571 available from Edmund Industrial Optical of Barrington, NJ. Moving plate 162 also provides a mounting base for camera 135. Preferably, camera 135 is a high-resolution monochrome megapixel CCD camera model # CVM1 from JAI America INC. of Laguna Hills, CA. Camera 135 has lens 145. Preferably, lens 145 has a 2.5x to 10x zoom capability and is model # NT54-396 available from Edmund Industrial Optical of Barrington, NJ.

Moving plate 162 also provides a mounting base for a zoom lens motor 192, an intelligent self-contained servo motor model # SM2315D available from Animatics Corp. of Santa Clara, CA., with 20 oz-in of available torque. The servo motor communicates with the Windows Based computer 105 through a serial connection routed through the motion control box 190. The zoom lens motor operates the zoom lens 145 through a conventional belt drive.

A bar-code reader 175 is mounted adjacent linear actuator 115 and is attached to support 172 fixed on granite top 170. Bar-code reader 175 is positioned to view a bar-code identity label attached to a micro-well plate when the micro-well plate is

positioned under linear actuator 150. Preferably, bar-code reader 175 is model # BL601 available from Keyence Corporation of America of Newark, NJ.

A plate sensor transmitter/receiver 186 is also fixed to support 172 and is aligned to sense whenever a micro-well plate 125 breaks a beam of light emitted by transmitter/receiver 186 and reflected by a reflector 188. Reflector 188 is mounted on support 191 on the opposite side of the linear actuator 115. Plate sensor transmitter/receiver 186 and reflector 188 are preferably model # E3T-SR13 available from Western Switch Controls of Santa Ana, CA.

#### Block Diagram Showing Connectivity of the Prototype

FIG. 6 shows a block diagram illustrating the connectivity of Applicant's prototype. Linear actuator motors 110, 120 and 130, and zoom motor 192 receive DC power from 48 Volt DC power supply 680 through an electrical connection. Linear actuator motors 110, 120 and 130, and zoom motor 192 motors communicate with Windows-based computer 105 through common serial line 633. Bar-code reader 175 communicates with computer 105 through a communications line and plate sensor 186 communicates with the computer 105 through a communications line. Monitor 620 displays information to the operator transmitted from computer 105. Cameras 135 and 155 communicate with frame grabber 630 through communication lines. Frame grabber 630 is installed within computer 105 and is preferably PCVision from Coreco Imaging US Office in Bedford, MA.. Frame grabber 630 digitizes the image from the camera to form the digitized image data array within computer 105. A 4-port Ethernet hub 640 provides for connectivity between computer 105, central control unit 190, and an external Ethernet 670. By providing for connectivity to the Ethernet, computer network communications are possible. The central control unit 190 controls light source 194 through an analog control line. Central control unit 190 receives 24 volt DC power from 24 volt DC power supply 660. Emergency stop button and switch (e-stop) 650 is connected to central control unit 190.

#### Experimental Results

Fifty-three test images were obtained from the system and were both automatically classified by the system and were manually classified by four scientists. Table 4



shows the correlation percentage between the various scientists and the automatic classification provided by the system.

Table 4

	Sam	Mary	Susan	Fred	Auto
Sam	100%				
Mary	98%	100%			
Susan	93%	97%	100%		
Fred	89%	93%	96%	100%	
Auto	95%	93%	86%	81%	100%

#### Utilization of Color

In another preferred embodiment, the present invention is configured to record color images. It is desirable to be able to analyze color images in that certain precipitation products in the protein crystallization process have distinctive colors and a crystallographer or automated image analysis algorithm may use the color information to help discriminate crystallization results.

#### True Color Picture

FIG. 37 shows a side view of micro-well plate 125A positioned on fixture plate 129. Support 191 with embedded light source 194 is positioned to the side of fixture plate 129. Light from light guide 195 is directed upward through cutout 131. Light guide 195 is positioned between fixture plate 129 and plate 127 such that both plates can move around the light guide 195 without interference.

Linear polarized filter 352 is rotationally mounted above light guide 195 such that light from light guide 195 can be polarized linearly at a programmable angle before it transits through micro-well plate 125A. Polarizer drive belt 356 (top view shown in FIG. 38) rotates polarized filter 352 about a vertical axis. Polarization drive belt 356 is driven by motor 358. Motor 358 is controlled by CCU 190 (FIG. 40). Second filter 354 (top view shown in FIG. 39) is positioned above micro-well plate 125A such that light transiting through micro-well plate 125A goes through second filter 354 before it goes into the camera zoom lens 145. Second filters 354 are mounted on

filter wheel apparatus 355. Filter wheel apparatus 355 rotates the operator selected second filter 354 into position under the zoom lens 145. The selected second filter 354 is preferably either a red, green or blue dichroic filter. Preferably, individual images taken through the red, green and blue second filters are combined to form a true color image.

#### False Color Image

In addition to the true color images that may be formed using red, green, and blue filters 354, a false color (also called a pseudo color) image may be formed by taking three individual images using linear polarized filter 352 at three different polarization angles with respect to a second filter. In this preferred embodiment, a linear polarized filter is substituted for the dichroic second filters 354 discussed above. For example, the polarized axis may be at 90 degrees to each other, and at plus and minus 45 degrees to each other. The three images are then called red, green, and blue and a false color image is produced. If the crystal exhibits any polarization rotation effects, then a very colorful image results. This pseudo color image is useful in detecting very small and fine crystals from the image background material. Other polarizing angles may be selected as well.

In the preferred embodiment, light guide 195 is model # A08925 fiber-optic backlight available from Aegis Electronics of Carlsbad, CA. Light source 194 is a Dolan-Jenner Model-PL-900 available from Edmund Industrial Optics, Barrington, NJ. First polarized filter 352, second filter 354 (including the linear polarized filters, and the dichroic red, green, blue filters) are available from Edmund Industrial Optics, Barrington, NJ. Filter wheel 355 is model FW1 available from Integrated Scientific Imaging Systems, Inc of Santa Barbara, CA.

#### Combined with an Automated Tray Storage and Retrieval System

In another preferred embodiment, proteomic crystal verification and inspection system 100 (FIG. 30) is operated in conjunction with automated storage and retrieval system 900, as shown in FIGS. 41A and 41B. FIG. 41A shows a front view and FIG. 41B shows a back view of the prototype automated storage and retrieval system 900 that Applicant's have built and tested. Also shown are two proteomic crystal verification and inspection systems 100. In the preferred embodiment, an operator

loads micro-well plate holding tray 901a (FIG. 42) into access drawer 902 (FIG. 41A). Preferably, micro-well plate holding tray 901a (FIG. 42) holds 6 micro-well plates that contain solution in which protein crystals are growing. Storage gantry 903 (FIG. 41B) removes tray 901a from access drawer 902. As shown in FIGS. 41A and 41B, automated storage and retrieval system 900 has a large number of storage slots. After removing tray 901a, storage gantry 903 transfers tray 901a to one of the storage slots. Preferably, this process is repeated until a plurality of micro-well plate holding trays are stored in a plurality of storage slots. Then, at defined time intervals, storage gantry 903 sequentially transfers micro-well plate holding trays from the storage slots to work cell area 904. An enlarged perspective view showing work cell area 904 is shown in FIG. 43. Once a micro-well plate holding tray has been placed in work cell area 904, work cell gantry 905 will sequentially remove micro-well plates from the tray and place them onto either one of the two proteomic crystal verification and inspection systems 100. Each well in the micro-well plates will then be inspected in a fashion similar to that described in the above preferred embodiments. After the micro-well plates have been inspected, work cell gantry 905 will place the micro-well plates back into their micro-well plate holding trays. Then, storage gantry 903 will return the micro-well plate holding trays back to their storage slots.

### Sequence of Operation

#### Storing the Microwell-Plates

FIG. 45 shows a front view similar to the perspective view shown in FIG. 41A. Access drawer 902 is closed.

FIG. 46 shows a top view of access drawer 902. In FIG. 46, an operator has opened access drawer 902.

In FIG. 47, the operator has placed trays 901a and 901b onto access drawer 902. Trays 901a and 901b each contain six 96-well micro-well plates. Inside each well of each micro-well plate is a solution in which the operator is attempting to grow protein crystals.

In FIG. 48, the operator has closed access drawer 902. Sensors 922a and 922b are situated adjacent to drawer 902 are directed to sense the presence of a tray on access

drawer 902. In the preferred embodiment, sensors 922a and 922b are MC-S series safety interlock switch, control unit and sensors manufactured by Scientific Technologies Inc. with offices in Fremont, CA. Upon sensing the presence of trays 901a and 901b, sensors 922a and 922b send signals to CPU 921 (FIG. 44). CPU 921 is a windows based computer that has been programmed to operate automated storage and retrieval system 900.

FIG. 49 shows a back view of automated storage and retrieval system 900 similar to the perspective view shown in FIG. 41B. In FIG. 49, trays 901a and 901b are resting on access drawer.

In FIG. 50, CPU 921 (after having received the signals from sensors 922a and 922b) has sent a signal to linear actuator motor 923a (FIG. 44). Linear actuator motor 923a is connected to linear actuator 923b directly and to linear actuator 923c through actuator drive shaft 923d. In the preferred embodiment, linear actuators 923b and 923c are belt driven linear actuators, Series HLE manufactured by Parker Automation- Daedal Division with offices in Irwin, PA. In FIG. 50, linear actuators 923b and 923c have moved storage gantry 903 to the left above tray 901a.

In FIG. 51, CPU 921 has sent a signal to linear actuator motor 924a. Linear actuator motor 924a is connected directly to belt driven linear actuator 924b. In the preferred embodiment, linear actuator 924b is Series HLE manufactured by Parker Automation- Daedal Division with offices in Irwin, PA. Linear actuator 924b controls the vertical motion of platform 925. Platform 925 is also supported by support bearing 924c. In FIG. 51, linear actuator 924b has lowered platform 925 so that is approximately adjacent to tray 901a. Note that as platform 925 has lowered, counterweight cylinder 926 has risen. Counterweight 926 rides on bearing 927 and is connected to platform 925 by cable 928. Cable 928 runs through pulleys 929a and 929b. In the preferred embodiment platform 925 weights approximately 70 lbs. (fully loaded) and counterweight 926 weights approximately 50 lbs. By connecting counterweight 926 to platform 925, linear actuator 924b has to do less work to move platform 925. Therefore, the vertical movement of platform 925 is smoother.

FIG. 52 shows a top view of the position of platform 925 in FIG. 51. Linear actuator motor 930a is connected directly to linear actuator 930b. Linear actuator motor 930a and linear actuator 930b are both supported by platform 925. In the preferred embodiment, linear actuator 930b is a gear driven linear actuator, ER Ball-screw series manufactured by Parker Automation- Daedal Division with offices in Irwin, PA. Linear actuator 930b controls the horizontal movement of robotic gripper motor 931a and robotic gripper 931b. Robotic gripper motor 931a controls robotic gripper 931b.

In FIG. 53, CPU 921 (FIG. 44) has sent a signal to linear actuator motor 930a, causing linear actuator 930b to move robotic gripper 931b closer to tray 901a. Robotic gripper has gripped tray 901a.

In FIG. 54, robotic gripper 931b has pulled tray 901a along tracks 933 onto platform 925. Bar code reader 934 is attached to the side of platform 925 and is orientated so that it is able to read the bar code of each micro-well plate as it passes by. The bar code information is transmitted to CPU 921 where it is stored in the computer's database (FIG. 44).

In FIG. 55, storage gantry 903 has moved tray 901a to its storage slot in storage rack 932. While at the location shown in FIG. 55, linear actuator motor 930a moves robotic gripper 931b (FIG. 52) towards storage rack 932 so that tray 901a is stored inside its storage slot.

In FIG. 56, after having deposited tray 901a in its storage slot, storage gantry 903 has moved adjacent to tray 901b.

In FIG. 57, storage gantry 903 has moved trays 901a – 901aab to their assigned storage slots in storage rack 932 in a fashion similar to that describe above in reference to FIGS. 44 – 56. Although FIG. 57 shows just fifty-four trays actually in storage rack 932, in the preferred embodiment, storage rack 932 can hold a total of 1692 trays. Also, in the preferred embodiment, each tray can hold six micro-well plates. Therefore, in the preferred embodiment, storage rack 932 can hold a total of 10,152 micro-well plates. Moreover, as previously noted in reference to FIG. 54, CPU 921 records the bar code of each micro-well plate as its being loaded onto platform 925. Also, in the preferred embodiment, CPU 921 records the location of

the storage slot in which each micro-well plate has been located. Therefore, for example, an operator can immediately ascertain the location of a particular micro-well plate by referring to the database of CPU 921.

#### Moving the Micro-well Plates to the Work Cell Area

In the preferred embodiment, by inserting commands into CPU 921 via keyboard 934 (FIG. 44) an operator can inspect any given micro-well plate at any moment. Or, CPU 921 can be programmed to automatically cause automated storage and retrieval system 900 (FIG. 58) to transfer a designated micro-well plate to proteomic crystal verification and inspection systems 100 at a specific designated time or at designated time intervals. For example, in a preferred embodiment, CPU 921 has been programmed to cause micro-well plates stored on trays 901a – 901d to be inspected every two weeks for protein crystal growth without any operator intervention.

In FIG. 58, storage gantry 903 has moved to the top of tray stack 935 containing trays 901a – 901aab. While at this location, micro-well plate 901a will be loaded onto platform 925 in a fashion similar to that described above.

In FIG. 59, storage gantry 903 has moved platform 925 containing micro-well plate 901a in front of work cell area 904.

FIG. 60 shows a top view of platform 925 in front of work cell area 904. Work cell area 904 contains platform 936. At the end of platform 936 are robotic grippers 937b having robotic gripper motors 937a. Robotic gripper motors 937a are controlled by CPU 921 (FIG. 44). Adjacent to platform 936 are fixture plates 129 of proteomic crystal verification and inspection systems 100. A preferred proteomic crystal verification and inspection system 100 is discussed above and shown in greater detail in FIG. 30. Work cell gantry 905 surrounds work cell area 904.

In FIG. 61, linear actuator 930b has moved tray 901a onto platform 936. Robotic gripper 937b has gripped the forward end of tray 901a

In FIG. 62, robotic gripper 924b has released the back end of tray 901a so that tray 901a has been left in position on platform 936.

In FIG. 63, trays 901b, 901c and 901d have been stored on platform 936 in a fashion similar to that described in reference to tray 901a.

In FIG. 64, linear actuator 938b of work cell gantry 905 has repositioned linear actuator 939b. Linear actuator motor 938a is connected to linear actuator 938b directly and to linear actuator 938d through actuator drive shaft 938c. In the preferred embodiment, linear actuators 938b and 938d are belt driven linear actuators, model number Series HLE manufactured by Parker Automation- Daedal Division with offices in Irwin, PA.

In FIG. 65, linear actuator 939b has moved gripping device 941 over a micro-well plate stored on tray 901a. Linear actuator 939b is controlled by linear actuator motor 939a. In the preferred embodiment, linear actuator 939b is a gear driven linear actuator, ER Ball-screw Series manufactured by Parker Automation- Daedal Division with offices in Irwin, PA.

FIG. 66 shows a side view of the situation in work cell area 904 shown in FIG. 65. Gripping device 941 is positioned over a micro-well plate stored on tray 901a. Gripping device 941 has linear actuator 942b controlled by linear actuator motor 942a. In the preferred embodiment, linear actuator 942b is a gear driven linear actuator, ET Ball-screw Series manufactured by Parker Automation- Daedal Division with offices in Irwin, PA. Linear actuator 942b controls the vertical movement of robotic gripper 943b. The gripping movement of robotic gripper 943b is controlled by robotic gripper motor 943a.

In FIG. 67, linear actuator 942b has lowered robotic gripper 943b towards tray 901a. Gripper 943b has gripped the micro-well plate on tray 901a.

In FIG. 68, gripper 943b has removed the micro-well plate from tray 901a.

In FIG. 69, linear actuator 939b has moved robotic gripping device 941 so that the micro-well plate is positioned over fixture plate 129.

In FIG. 70, gripper 943b has lowered the micro-well plate onto fixture plate 129.

In FIG. 71, gripper linear actuator 942b has raised gripper 943b, leaving behind the micro-well plate on fixture plate 129.

FIG. 72 shows a top view of the situation depicted in FIG. 71. Robotic gripping device 941 is positioned over fixture plate 129.

In FIG. 73, all the micro-well plates stored on tray 901a have been transferred to fixture plate 129.

In FIG. 74, fixture plate 129 is moving the micro-well plates underneath lens 944 of proteomic crystal verification and inspection system 100. The operation of fixture plate 129 and proteomic crystal verification and inspection systems 100 is described in detail above in reference to FIGS. 1 – 40. Robotic gripping device 941 has moved so that it is depositing a micro-well plate stored on tray 901d onto the second fixture plate 129.

In FIG. 75, first fixture plate 129 has continued sequentially moving micro-well plates under lens 944. Robotic gripping device 941 is in the process of depositing micro-well plates on second fixture plate 129.

In FIG. 76, second fixture plate 129 is sequentially moving micro-well plates under lens 944. Robotic gripping device 941 is in the process of removing micro-well plates from first fixture plate 129 and placing them back onto tray 901a.

In FIG. 77, all micro-well plates on trays 901a through 901d have been inspected by proteomic crystal verification and inspection system 100 in the manner described above. All micro-well plates are now back on trays 901a through 901d. Storage gantry 903 (FIG. 41B and FIG. 49) will now move trays 901a through 901d back to their original positions in storage rack 932 (FIG. 57) until they are scheduled to be inspected again in accordance with programming input into CPU 921 (FIG. 44).

#### Operator Retrieval of Stored Tray



In the preferred embodiment, an operator has the option of personally inspecting or handling a micro-well plate that has been stored in storage rack storage rack 932 (FIG. 55). The operator first inputs the micro-well plate's identifying bar code into CPU 921 via keyboard 934 (FIG. 44). CPU sends a signal to storage gantry 903 (FIG. 41B, FIG. 59) and the appropriate tray containing the requested micro-well plate is removed from storage rack 932. Storage gantry 903 then delivers the tray containing the requested micro-well plate to access drawer 902 (FIG. 41A, FIG. 46). The tray is loaded onto the access drawer 902. Once the tray is on access drawer 902, the operator can personally retrieve the tray by opening the drawer, as shown in FIG. 46. After he is finished with the tray, the operator returns the tray by placing it back on access drawer 902. Tray sensors 922a and 922b (FIG. 48) will sense the presence of the tray and CPU will send a signal to storage gantry 903 to grip the tray. Bar code reader 934 (FIG. 54, FIG. 44) will read the bar code of the micro-well plates on the tray and storage gantry 903 will return the tray to its appropriate storage slot in storage rack 932.

#### Preferred Micro-well plate Holding Tray

Although a variety of designs for tray 901a will work in conjunction with the present invention, a preferred micro-well plate holding tray 901a can be seen by reference to FIGS. 78 – 82. FIG. 78 shows a perspective view of a preferred micro-well plate holding tray 901a with a left-rail 906a, a top-rail 906b, a right-rail 906c, and a bottom-rail 906d. In the preferred embodiment the rectangular dimensions of tray 901a are approximately 58 mm by 148 mm. The rails are preferably designed to be at or below the height of a typical micro-well plate. In the preferred embodiment, they are approximately 14 mm in height. Also, in the preferred embodiment, tray 901a can store six micro-well plates. The storage tray is molded in one-piece of thermoplastic resin and can be clear or colored. Color coding of the trays adds visual feedback for sample identification. Six rectangular pockets 907a – 907f (each one designed to hold a standard micro-well plate) are formed in tray 901a, between rails 906a – 906d and interior rails 908a – 908d. Cut-down area 909 centered in top-rail 906b provides access for a robotic gripper. For example, a robotic gripper can grasp top rail 906b at cut-down area 909 and pull it or push it from or into one of the storage slots shown in automated storage and retrieval system 900 (FIGS. 41A and 41B). Thermoplastic tray

901a is smooth on its bottom side and can therefore efficiently slide over supporting surfaces so that the samples contained within the micro-well plate wells are not subject to adverse jarring or adverse acceleration. Top-left-side corner flat 910 provides orientation reference for tray 901a. Rounded top-right-side corner 912 is similar to the other two corners on bottom-rail 906d of tray 901a. Four second-cut-down areas 911a – 911d provide robotic access to allow for unstacking and stacking of the trays.

FIG. 79 is an enlarged partial perspective front-side view of tray 901a showing pocket 907a formed between top rail 906b, interior rail 908a, left rail 906a and right rail 906c and having bottom support 913. The preferred internal dimensions of the pockets between the enclosing rails are approximately 90 mm by 142 mm. Planar bottom support 913 has rectangular recess forming a bottom-plate-shelf 914. Opening 915 provides for structural flatness of the 901a in the molding process. Four robotic access cut-outs 916a – 916d, allow a robotic gripper to extend the horizontal plane of bottom-plate-shelf 914 and grasp a micro-well plate within pocket 907a. For example, robotic grippers on work cell gantry 905 can grab a micro-well plate in tray 901a and place it on either of the two proteomic crystal verification and inspection systems 100, shown in FIG. 43. Eight tapered guide pillars 917a – 917h provide for centering and guiding a micro-well plate into 901a when the micro-well plate is placed into the tray. The tapered guide pillars 917a – 917h have their side facing the interior of the pocket sloped at preferably 5 degrees away from normal to the planar bottom-support 913 such that as the micro-well plate is placed within the pocket, the tapers help to center the plate as it is set onto the recessed bottom-plate shelf 914. The guiding features of pillars 917a – 917h work together with the access cutouts 916a – 916d to allow easy robotic storing and retrieval of the plates, even though micro-well plates may have slightly different dimensions from manufacturer to manufacturer.

FIG. 80 shows an enlarged cross-sectional view of section A-A of FIG. 79 showing the planar bottom support 913 with its rectangular recess forming a bottom-plate-shelf 914. A second shelf 918b is formed in the rim of the rails of the tray such that groove 918a formed in the back-side of tray 901a will locate and properly nest into the second shelf 918b of a second tray when two or more trays are stacked together, one on top of the other.

FIG. 81 shows a bottom view of tray 901a showing the six open areas, corner flat 910, rounded corner 912, robotic access cut-outs 916a – 916x, and groove 918a for nesting of trays.

FIG. 82 is an enlarged partial perspective view of tray 901a holding a 96 well micro-well plate 919 and positioned to illustrate the visibility of bar code 220 for a bar-code identifier for the plate. A properly positioned external bar-code reader may read the barcode of the micro-well plate while the plate is contained within the pocket in the tray. The cut-down area 909 in the top-rail of the tray provides access for a robotic tray gripper. The top-left-side corner flat 910 provides orientation reference for the tray. The rounded top-right-side corner 912 is similar to the other two corners on a bottom side of the tray.

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Although the above-preferred embodiments have been described with specificity, persons skilled in this art will recognize that many changes to the specific embodiments disclosed above could be made without departing from the spirit of the invention. For example, although in the above preferred embodiments automated storage and retrieval system 900 was shown as being used in conjunction with the automated receiving machine identified as proteomic crystal verification and inspection system 100, automated storage and retrieval system 900 could be used to store and retrieve a variety of subject matter for a variety of purposes other than proteomic crystal verification and inspection. For example, in another preferred embodiment, the two protein crystal verification and inspection systems 100 could be replaced with two other automated receiving machines. In one preferred embodiment, the two protein crystal verification and inspection systems 100 are replaced with two automated micro-well plate filling machines similar to the one disclosed in Applicant's U.S. Patent Application Serial No. 09/702,164. In this preferred embodiment, storage gantry 903 removes trays containing empty micro-well plates from storage rack 932 and delivers the trays to work cell gantry 905. Work cell gantry 905 sequentially transfers the trays to the automated micro-well plate filling machines in a fashion similar to that described above in reference to the transfer of

trays to the protein crystal verification and inspection systems 100. After the micro-well plates have been filled, storage gantry 903 delivers the trays to storage rack 932 for storage. Also, although access drawer 902 was discussed in detail in the above preferred embodiments, it would also be possible to utilize other types of access devices other than the access drawer described for depositing and removing trays. For example, the above invention could be configured so that an operator can insert trays into automated storage and retrieval system 900 by placing them on a flat table. Storage gantry 903 would then remove them from the flat table and store the trays in storage rack 932. Also, although the above preferred embodiments discussed the utilization of two protein crystal verification and inspection systems 100, it would also be possible to utilize just one of the two protein crystal verification and inspection system 100. Also, although the above preferred embodiments showed preferred tray 901a being utilized with automated storage and retrieval system 900, it could also be used with a variety of other automated machines that have robotic grippers. Also, although the above preferred embodiments discussed work cell gantry 905 moving micro-well plates, work cell gantry 905 could also move other types of subject matter stored in trays other than micro-well plates. For example, work cell gantry 905 could also move pharmaceuticals, disposable health care products such as catheters and syringes, and biological compounds in micro-well plates or in individual bottles in storage trays, or the tray itself could be an individual micro-well plate handled directly by the gantry. In addition, though the present invention is described in terms of 6 micro-well plates to a tray, any number of micro-well plates could be handled with the appropriately sized tray, from one to 24 or more. Also, with regards to the proteomic crystal verification and inspection system, although the above preferred embodiments specifically describe an indexing device in which linear actuators 115, 150, and 160 operate in conjunction to sequentially position protein crystals under cameras 155 and 135, there are a variety of other types of robotic indexing devices that could also be utilized for the same purpose. For example, an indexing device could be built in which the plurality of micro-well plates are kept in a stationary position. The camera lens would be attached to an indexing device that is preferably capable of unrestricted movement in the horizontal plane. The camera lens would be moved sequentially from micro-well to micro-well in the horizontal plane. Once in position over a micro-well, the lens could be raised or lowered in the vertical direction to achieve proper zoom and focus. In another embodiment, an indexing

device could be built in which cameras 155 and 135 are kept stationary with respect to horizontal movement. In this embodiment, the plurality of micro-well plates would be preferably placed on a positioning platform that is capable of unrestricted movement in the horizontal plane. In this fashion, the positioning platform could be moved so that each micro-well is sequentially positioned underneath the appropriate camera. As with the previous embodiment, once in position over a micro-well, the lens could be raised or lowered in the vertical direction to achieve proper zoom and focus. Also, although the first preferred embodiment discussed inspecting crystals grown by the hanging drop method, other crystals grown utilizing other methods could be inspected with equal effectiveness. For example, FIG. 12 shows protein crystal growth as a result of aqueous drop in oil protein crystallization. Cameras 135 and 155 focus on the crystals in drop 362. Also, although the above preferred embodiments discussed in detail how the present invention is utilized for inspecting protein crystals inside drops of liquid, the present invention could also be utilized to inspect other types of microscopic specimens. For example, the present invention could be utilized to inspect typical micro-well micro titer plate reactions wherein the quality of the reaction can be judged by the amount and wavelength of fluorescence emitted by the specimen by configuring the system with appropriate light sources, filters, and sensitive cameras as is typical for fluorescence detection. . Also, although the above preferred embodiments disclosed the utilization of two cameras 135 and 155, it would also be possible to have just one camera that is capable of zooming out so that it can focus on the entire well and zooming in so that it can focus on the drop of liquid containing the crystal. In addition, although an area CCD camera is shown, a linear CCD camera combined with moving of the micro-well plate would also work in the present invention. Also, in another preferred embodiment the detents 510 and 520 can be simply spring loaded and not controlled by the computer 105. Also, the invention is taught with a light panel light source, and or a bulb, likewise LED light sources and laser light sources could also be used with the present invention. Although the system is shown that only moves the micro-well plates in one axis and the camera in the other two axes, the invention could likewise be practiced with either the micro-wells moving in two orthogonal axes (such as X and Y) while the camera moves only in the Z-axis or the motion of all three axes be done with the camera system, wherein the micro-well plates are stationary and the system moves above them. These other variations of system design could also require rearrangement of the

light source or multiple light sources. Also, other filter types may be substituted for second filter 354. For example, a linearly polarized filter would be very effective. Also, although the above preferred embodiments disclosed specific types of cameras 135 and 155, other CCD cameras may be used in the present invention with less resolution or with greater resolution and still practice the present invention. For example, cameras of 2,000 by 2,000 pixels and even 4,000 by 4,000 pixels are commercially available from several vendors. When digitizing these alternative cameras, the digitized image would have the corresponding resolution of the camera. Also, one may practice this invention and digitize to greater gray-scale accuracy than 8-bit and gain advantage if the camera supports the greater bit depth, for example if the camera were cooled to reduce image noise. Therefore, the attached claims and their legal equivalents should determine the scope of the invention.

We claim:

- 1) An automated storage and retrieval device for trays holding subject matter, comprising:
  - A) an access device for the insertion and removal of a plurality of trays,
  - B) a storage rack for storing said plurality of trays,
  - C) at least one automated receiving machine,
  - D) a work cell gantry, for moving said subject matter to and from said at least one automated receiving machine, and
  - E) a storage gantry for moving said plurality of trays between said access device, said storage rack and said work cell gantry,
  - F) at least one computer system programmed to control said storage gantry and said work cell gantry.
- 2) The automated storage and retrieval device as in Claim 1, wherein said access device is an access drawer.
- 3) The automated storage and retrieval device as in Claim 1, wherein said at least one automated receiving machine is a device for inspecting and classifying a plurality of microscopic crystals.
- 4) The automated storage and retrieval device as in Claim 1, wherein said at least one automated receiving machine comprises:
  - A) at least one camera,
  - B) an indexing device for sequentially placing said microscopic crystals in camera-view of said at least one camera, and
  - C) at least one control computer programmed to control said indexing device and said at least one camera, wherein said at least one control computer is programmed to receive from said at least one camera images of said plurality of microscopic crystals, wherein said at least one control computer is programmed to classify said plurality of microscopic crystals.
- 5) The automated storage and retrieval device as in Claim 4, wherein said at least one control computer automatically classifies said plurality of microscopic crystals after receiving said images.

- 6) The automated storage and retrieval device as in Claim 1, wherein said at least one automated receiving machine is an automated micro-well plate filling machine.
- 7) The automated storage and retrieval device as in Claim 1, wherein said at least one automated receiving machine comprises:
  - A) a micro-well plate filling assembly, comprising:
    1. an indexing device, and
    2. a fill mechanism in communication with a media source and positioned to insert portions of said media into the empty micro-well plates, and
  - B) an automatic control unit programmed to cause said indexing device to move empty micro-well plates adjacent to said fill mechanism, and to cause said fill mechanism to inject media from said media source into wells in the micro-well plates.
- 8) The automated storage and retrieval device as in Claim 1, wherein said subject matter is at least one micro-well plate.
- 9) The automated storage and retrieval device as in Claim 8, wherein said at least one micro-well plate comprises a bar code, wherein said automated storage and retrieval device further comprises at least one bar code reader in communication with said at least one computer system.
- 10) The automated storage and retrieval device as in Claim 1, wherein said plurality of trays holds at least one micro-well plate, wherein said storage gantry and said work cell gantry each comprise at least one robotic gripper, wherein said plurality of trays comprises:
  - A) at least one cut-down access area for said at least one robotic gripper,
  - B) a corner flat for tray orientation, and
  - C) a plurality of tapered guide pillars for guiding said at least one micro-well plate into said plurality of trays.
- 11) A tray for holding at least one micro-well plate, wherein said tray is handled by at least one robotic gripper, wherein said plurality of trays comprises:



- A) at least one cut-down access area for said at least one robotic gripper,
  - B) a corner flat for tray orientation, and
  - C) a plurality of tapered guide pillars for guiding said at least one micro-well plate into said tray.
- 12) A method for automatically storing and retrieving a plurality of trays, wherein said plurality of trays holds at least one micro-well plate, comprising the steps of:
- A) inserting said plurality of trays onto an access device,
  - B) transferring via said storage gantry said plurality of trays to a storage rack,
  - G) transferring via said storage gantry said plurality of trays to a work cell gantry,
  - H) transferring via said work cell gantry said at least one micro-well plate from said plurality of trays to at least one automated receiving machine,
  - I) transferring via said work cell gantry said at least one micro-well plate from said at least one automated receiving machine to said plurality of trays,
  - J) transferring via said storage gantry said plurality of trays to said access device,
  - K) removing said plurality of trays from said access device, and
  - L) controlling the movement of said storage gantry and said work cell gantry via at least one programmed computer system.
- 13) The method as in Claim 12, wherein said access device is an access drawer.
- 14) The method as in Claim 12, wherein said at least one automated receiving machine is a device for inspecting and classifying a plurality of microscopic crystals.
- 15) The method as in Claim 12, wherein said at least one automated receiving machine comprises:
- A) at least one camera,
  - B) an indexing device for sequentially placing said microscopic crystals in camera-view of said at least one camera, and
  - C) at least one control computer programmed to control said indexing device and said at least one camera, wherein said at least one control computer is programmed to receive from said at least one camera images of said plurality

of microscopic crystals, wherein said at least one control computer is programmed to classify said plurality of microscopic crystals.

- 16) The method as in Claim 15, wherein said at least one control computer automatically classifies said plurality of microscopic crystals after receiving said images.
- 17) The method as in Claim 12, wherein said at least one automated receiving machine is an automated micro-well plate filling machine.
- 18) The method as in Claim 12, wherein said at least one automated receiving machine comprises:
  - A) a micro-well plate filling assembly, comprising:
    1. an indexing device, and
    2. a fill mechanism in communication with a media source and positioned to insert portions of said media into the empty micro-well plates, and
  - B) an automatic control unit programmed to cause said indexing device to move empty micro-well plates adjacent to said fill mechanism, and to cause said fill mechanism to inject media from said media source into wells in the micro-well plates.
- 19) The method as in Claim 12, wherein said plurality of trays holds at least one micro-well plate.
- 20) The method as in Claim 19, wherein said at least one micro-well plate comprises a bar code, wherein said automated storage and retrieval device further comprises at least one bar code reader in communication with said at least one computer system.
- 21) The method as in Claim 12, wherein said plurality of trays holds at least one micro-well plate, wherein said storage gantry and said work cell gantry each comprise at least one robotic gripper, wherein said plurality of trays comprises:
  - A) at least one cut-down access area for said at least one robotic gripper,
  - B) a corner flat for tray orientation, and

- C) a plurality of tapered guide pillars for guiding said at least one micro-well plate into said plurality of trays.
- 22) An automated storage and retrieval device for trays holding subject matter, comprising:
- A) an access means for the insertion and removal of a plurality of trays,
  - B) a storage rack means for storing said plurality of trays,
  - C) at least one automated receiving machine means,
  - D) a work cell gantry means for moving said subject matter to and from said at least one automated receiving machine,
  - E) a storage gantry means for moving said plurality of trays between said access device, said storage rack and said work cell gantry, and
  - F) at least one computer system means programmed to control said storage gantry means and said work cell gantry means.
- 23) The automated storage and retrieval device as in Claim 22, wherein said access means is an access drawer.
- 24) The automated storage and retrieval device as in Claim 22, wherein said at least one automated receiving machine means is a device for inspecting and classifying a plurality of microscopic crystals.
- 25) The automated storage and retrieval device as in Claim 22, wherein said at least one automated receiving machine means comprises:
- A) at least one camera,
  - B) an indexing device for sequentially placing said microscopic crystals in camera-view of said at least one camera, and
  - C) at least one control computer programmed to control said indexing device and said at least one camera, wherein said at least one control computer is programmed to receive from said at least one camera images of said plurality of microscopic crystals, wherein said at least one control computer is programmed to classify said plurality of microscopic crystals.

- 26) The automated storage and retrieval device as in Claim 25, wherein said at least one control computer automatically classifies said plurality of microscopic crystals after receiving said images.
- 27) The automated storage and retrieval device as in Claim 22, wherein said at least one automated receiving machine means is an automated micro-well plate filling machine.
- 28) The automated storage and retrieval device as in Claim 22, wherein said at least one automated receiving machine means comprises:
- A) a micro-well plate filling assembly, comprising:
    - 1. an indexing device, and
    - 2. a fill mechanism in communication with a media source and positioned to insert portions of said media into the empty micro-well plates, and
  - B) an automatic control unit programmed to cause said indexing device to move empty micro-well plates adjacent to said fill mechanism, and to cause said fill mechanism to inject media from said media source into wells in the micro-well plates.
- 29) The automated storage and retrieval device as in Claim 22, wherein said subject matter at least one micro-well plate.
- 30) The automated storage and retrieval device as in Claim 29, wherein said at least one micro-well plate comprises a bar code, wherein said automated storage and retrieval device further comprises at least one bar code reader in communication with said at least one computer system means.
- 31) The automated storage and retrieval device as in Claim 22, wherein said plurality of trays holds at least one micro-well plate, wherein said storage gantry means and said work cell gantry means each comprise at least one robotic gripper, wherein said plurality of trays comprises:
- A) at least one cut-down access area for said at least one robotic gripper,
  - B) a corner flat for tray orientation, and

- C) a plurality of tapered guide pillars for guiding said at least one micro-well plate into said plurality of trays.
- 32) A tray for holding at least one micro-well plate, wherein said tray is handled by at least one robotic gripper, wherein said plurality of trays comprises:
- A) at least one cut-down access area means for said at least one robotic gripper,
  - B) a corner flat means for tray orientation, and
  - C) a plurality of tapered guide pillar means for guiding said at least one micro-well plate into said tray.
- 33) A method for growing protein crystals, comprising the steps of:
- A) inserting drops of protein solution into a plurality of micro-well plates,
  - B) placing said plurality of micro-well plates onto a plurality of trays,
  - C) inserting said plurality of trays onto an access device,
  - D) transferring via said storage gantry said plurality of trays to a storage rack,
  - E) transferring via said storage gantry said plurality of trays to a work cell gantry,
  - F) transferring via said work cell gantry said plurality of micro-well plates from said plurality of trays to an automated machine for inspecting and classifying a plurality of microscopic crystals,
  - G) analyzing via said automated machine said drops of protein solution,
  - H) transferring via said work cell gantry said plurality of micro-well plates from said at least one automated receiving machine to said plurality of trays,
  - I) transferring via said storage gantry said plurality of trays to said access device,
  - J) removing said plurality of trays from said access device, and
  - K) controlling the movement of said storage gantry and said work cell gantry via at least one programmed computer system.
- 34) Protein crystals grown by a method comprising the steps of:
- A) inserting drops of protein solution into a plurality of micro-well plates,
  - B) placing said plurality of micro-well plates onto a plurality of trays,
  - C) inserting said plurality of trays onto an access device,
  - D) transferring via said storage gantry said plurality of trays to a storage rack,
  - E) transferring via said storage gantry said plurality of trays to a work cell gantry,

- F) transferring via said work cell gantry said plurality of micro-well plates from said plurality of trays to an automated machine for inspecting and classifying a plurality of microscopic crystals,
- G) analyzing via said automated machine said drops of protein solution,
- H) transferring via said work cell gantry said plurality of micro-well plates from said at least one automated receiving machine to said plurality of trays,
- I) transferring via said storage gantry said plurality of trays to said access device,
- J) removing said plurality of trays from said access device, and
- K) controlling the movement of said storage gantry and said work cell gantry via at least one programmed computer system.

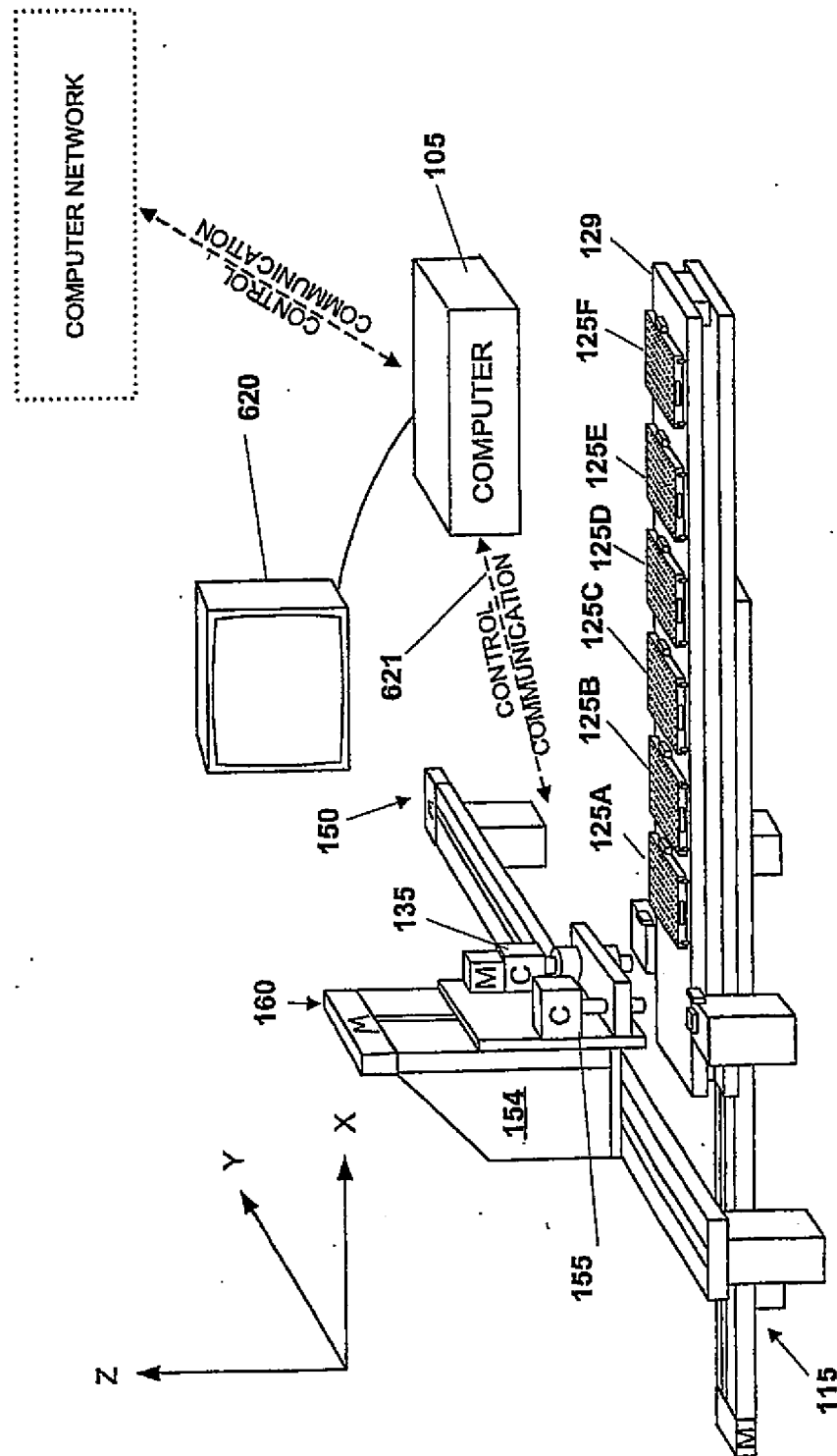


FIG. 1

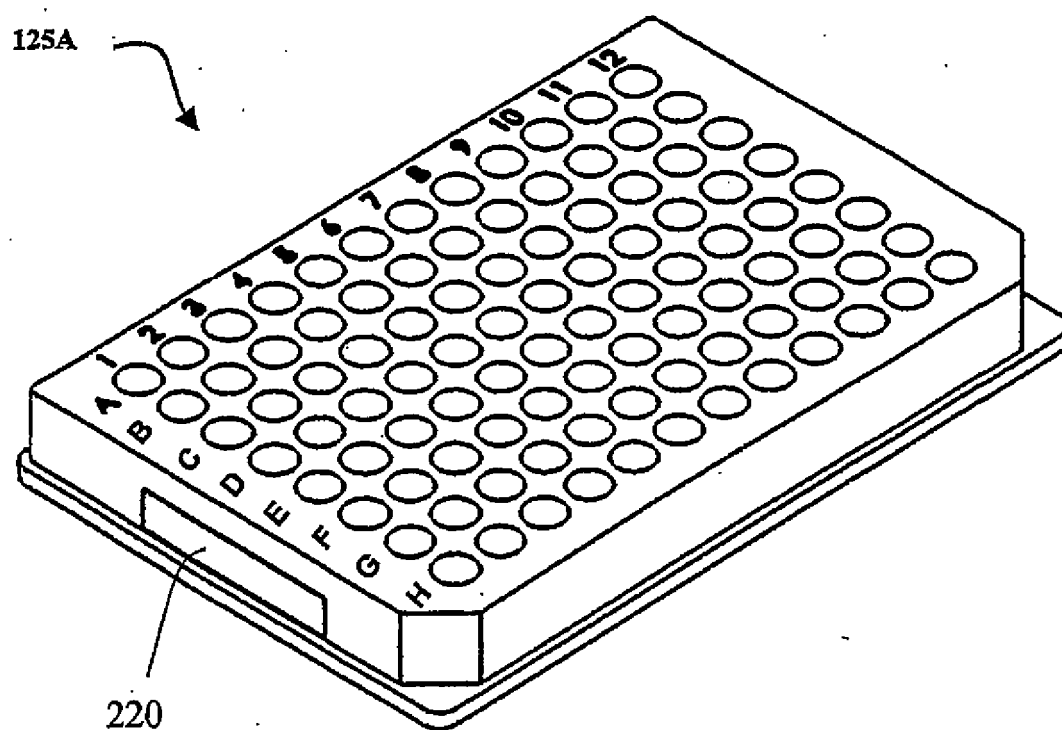


FIG. 2



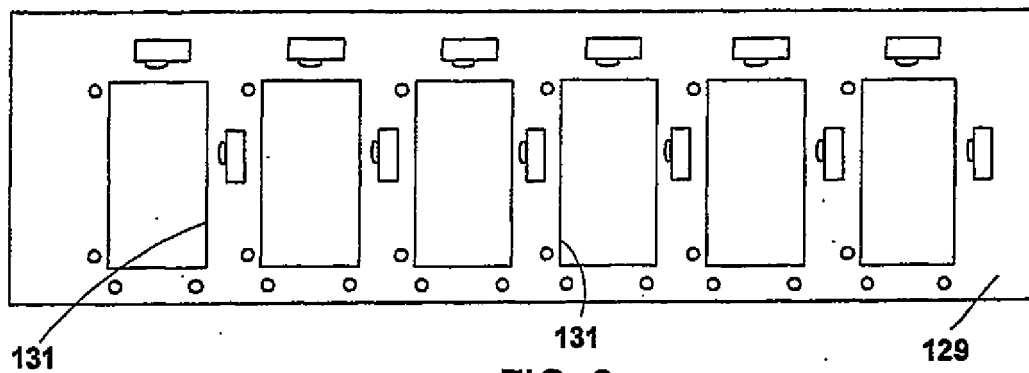


FIG. 3

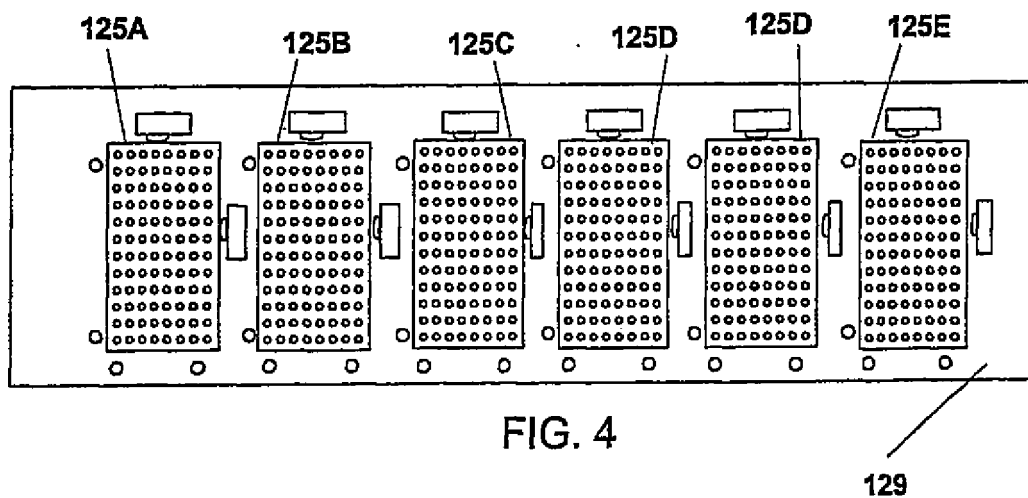


FIG. 4

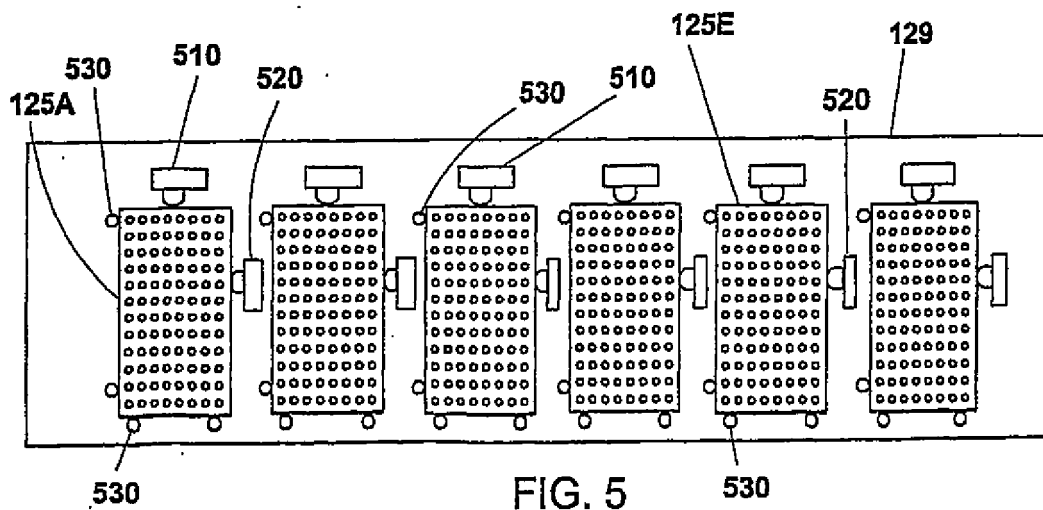


FIG. 5

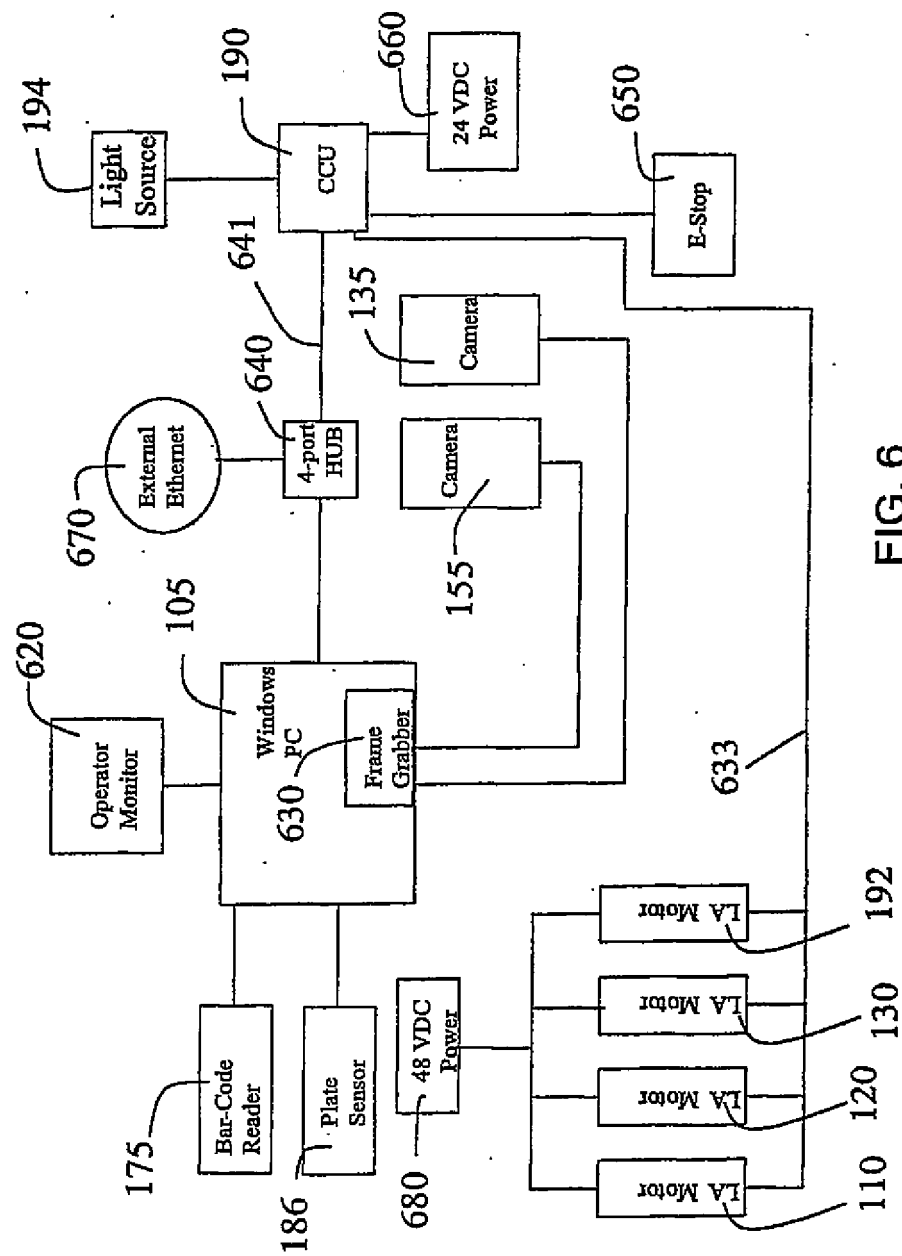


FIG. 6

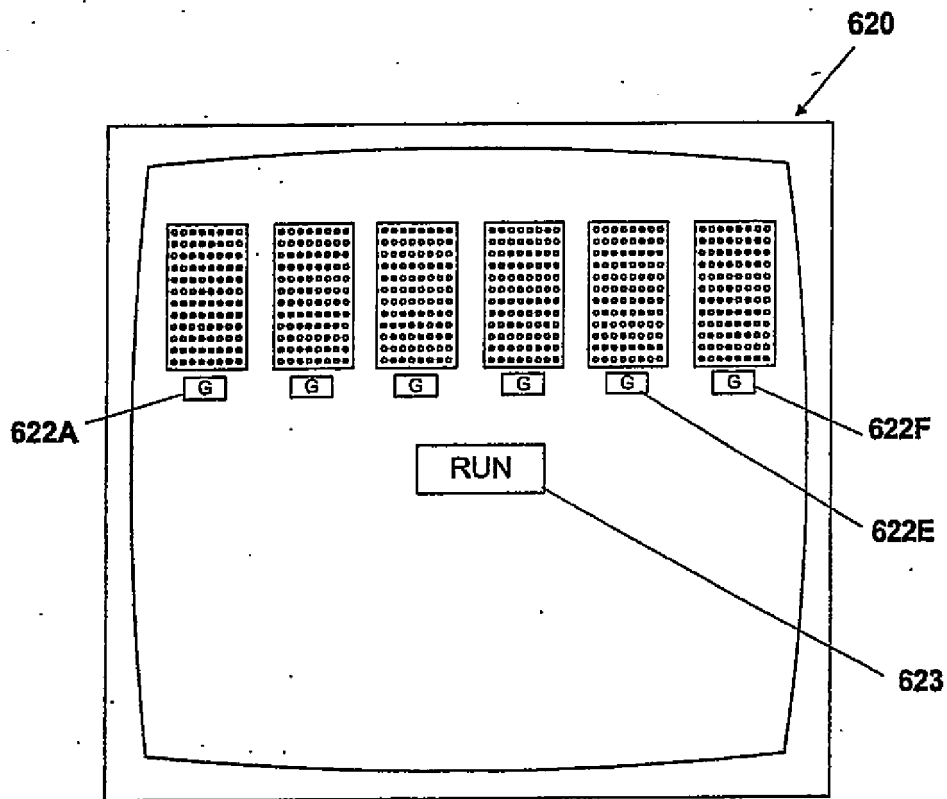


FIG. 7

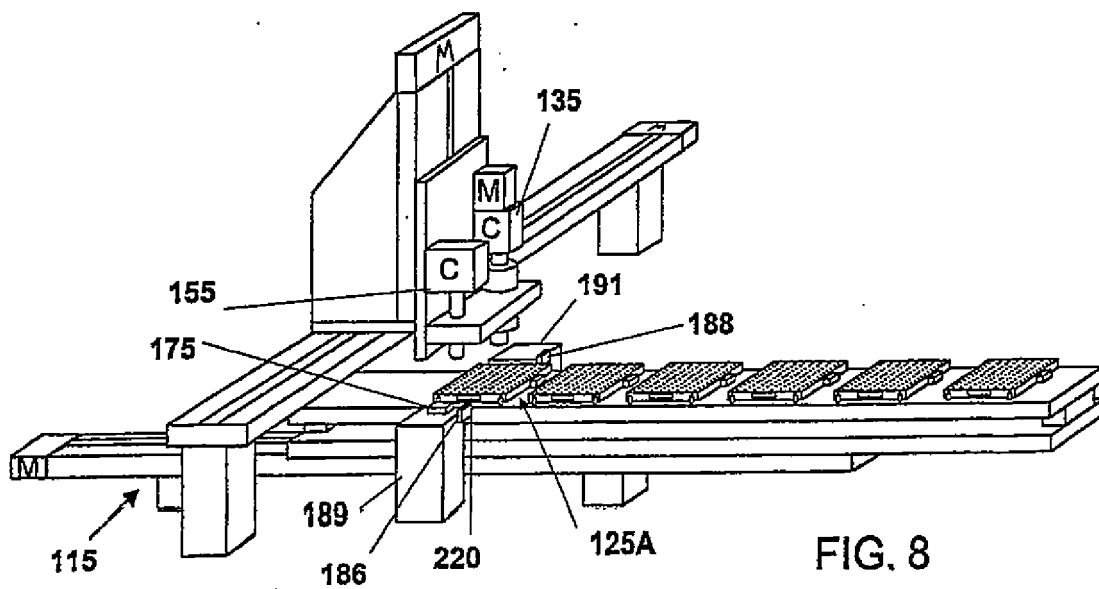
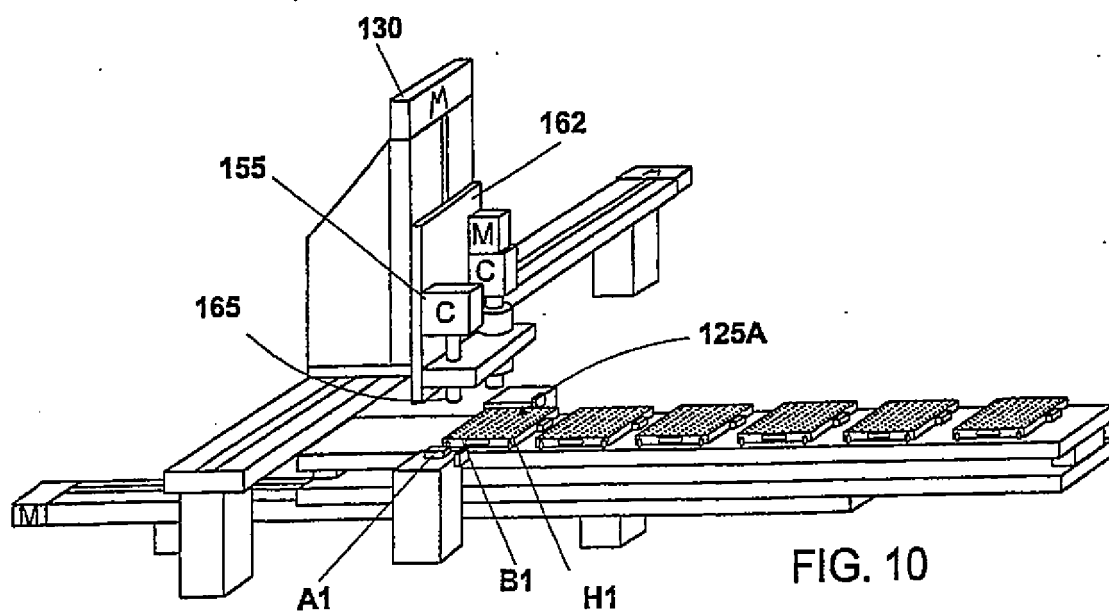
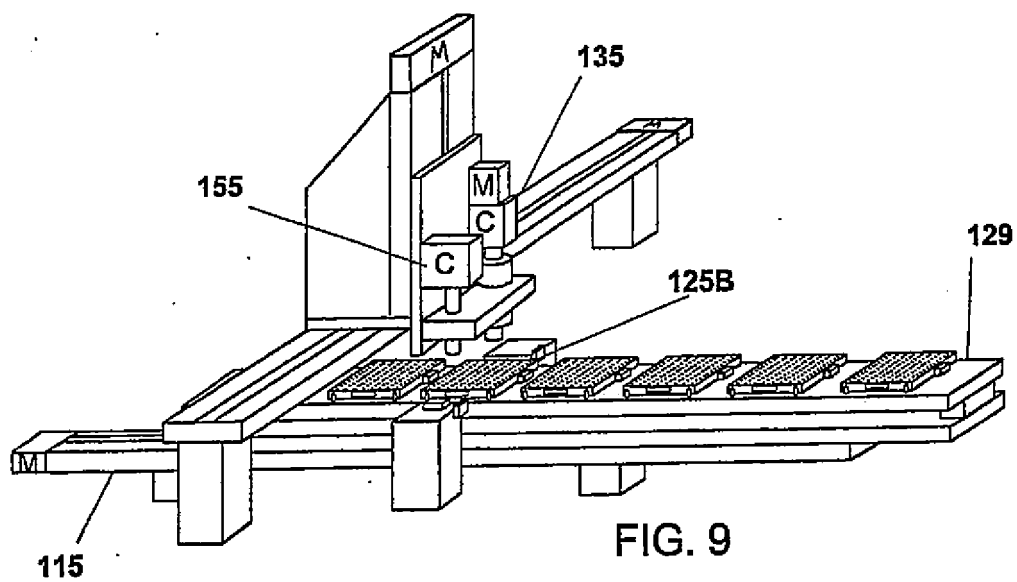


FIG. 8



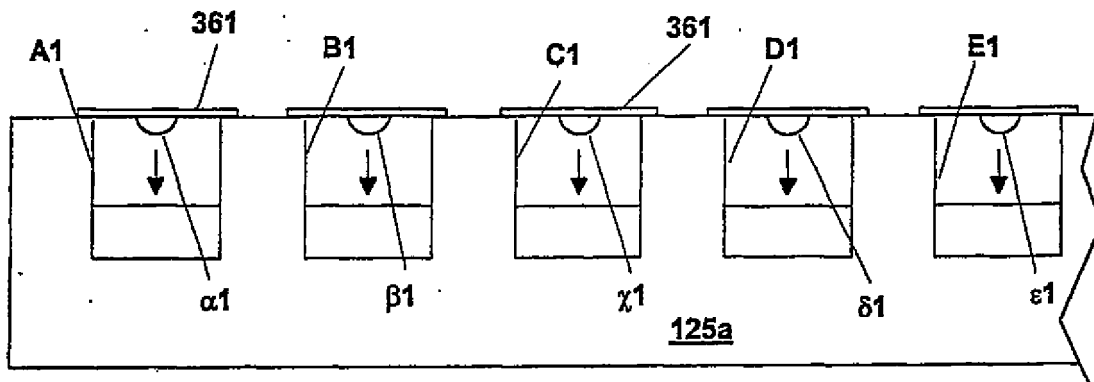


FIG. 11

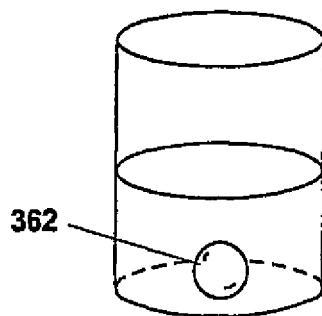


FIG. 12

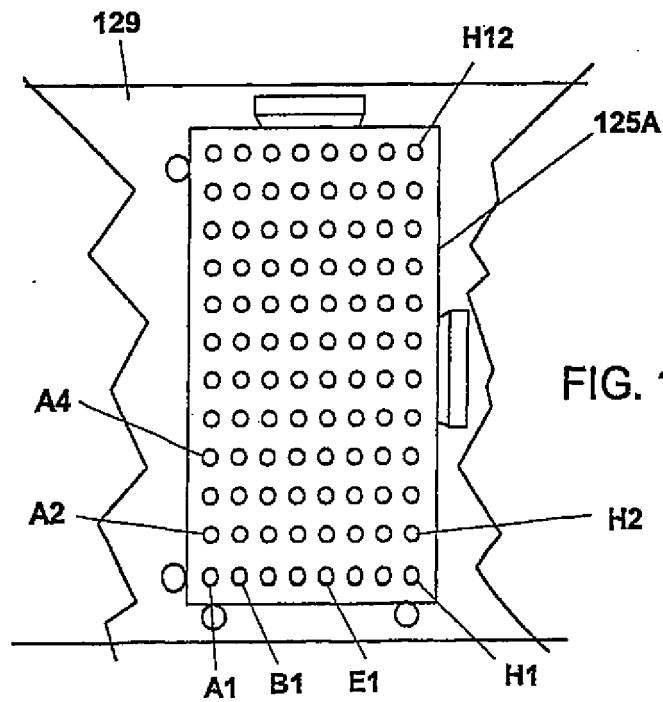


FIG. 13

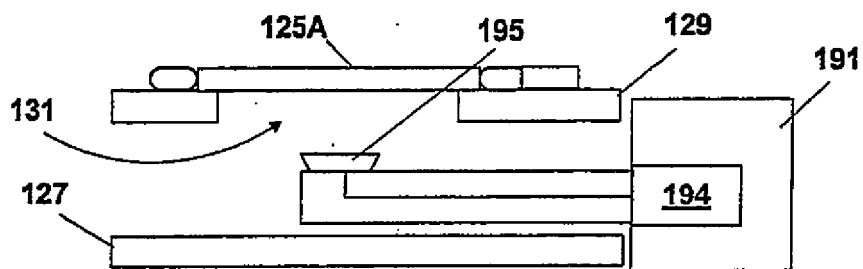


FIG. 14

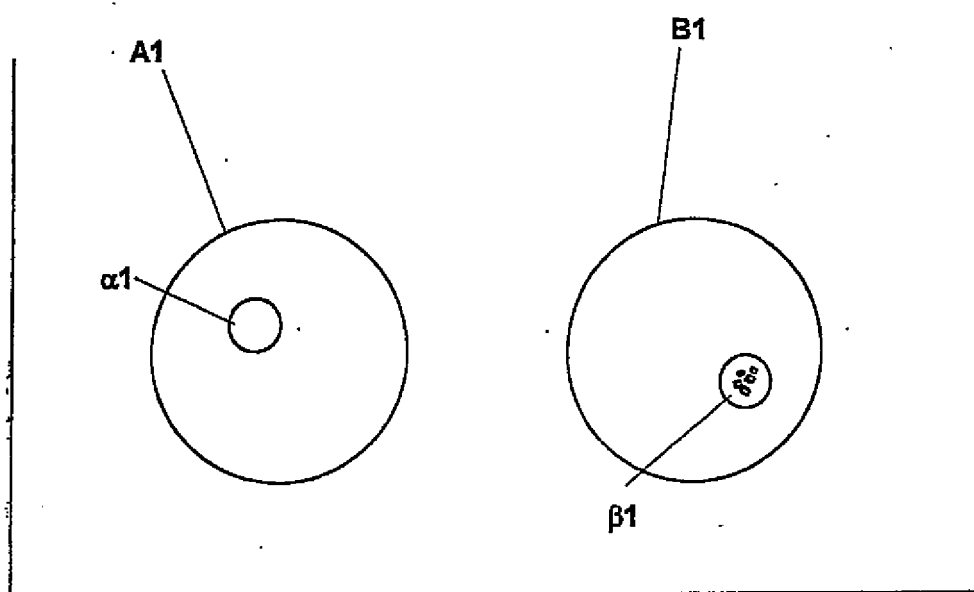


FIG. 15

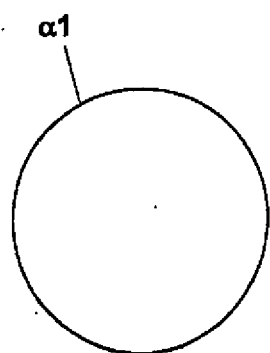


FIG. 16

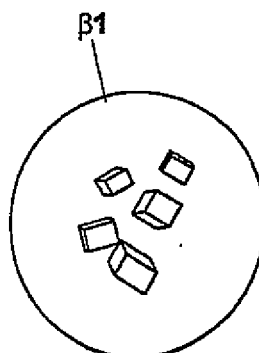
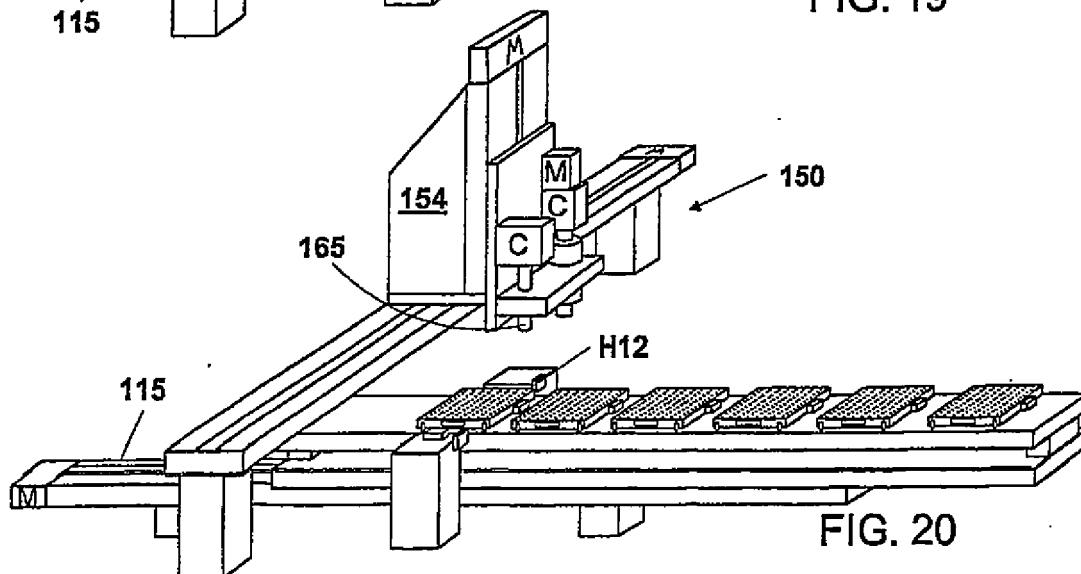
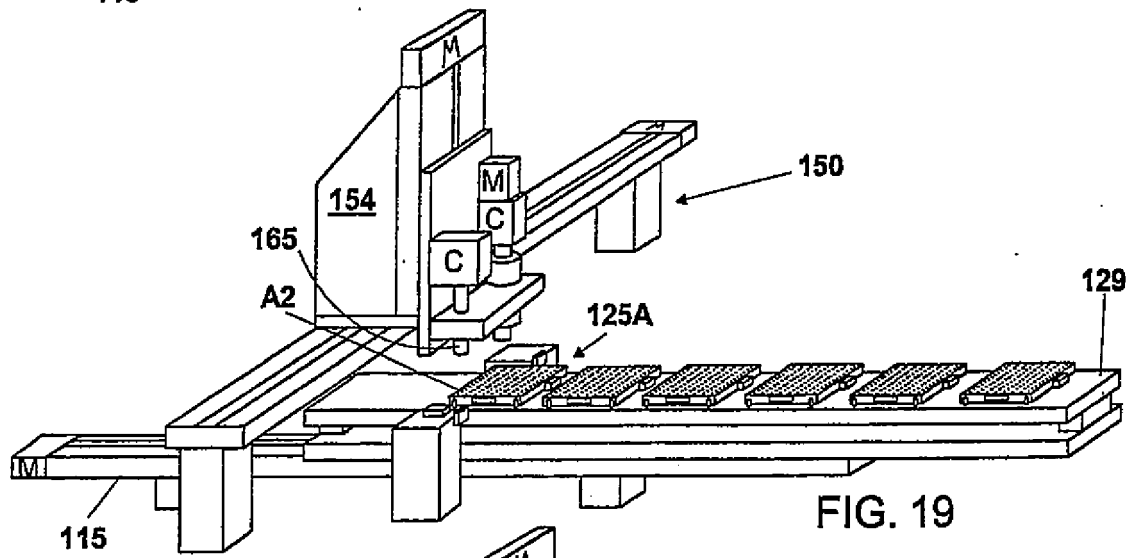
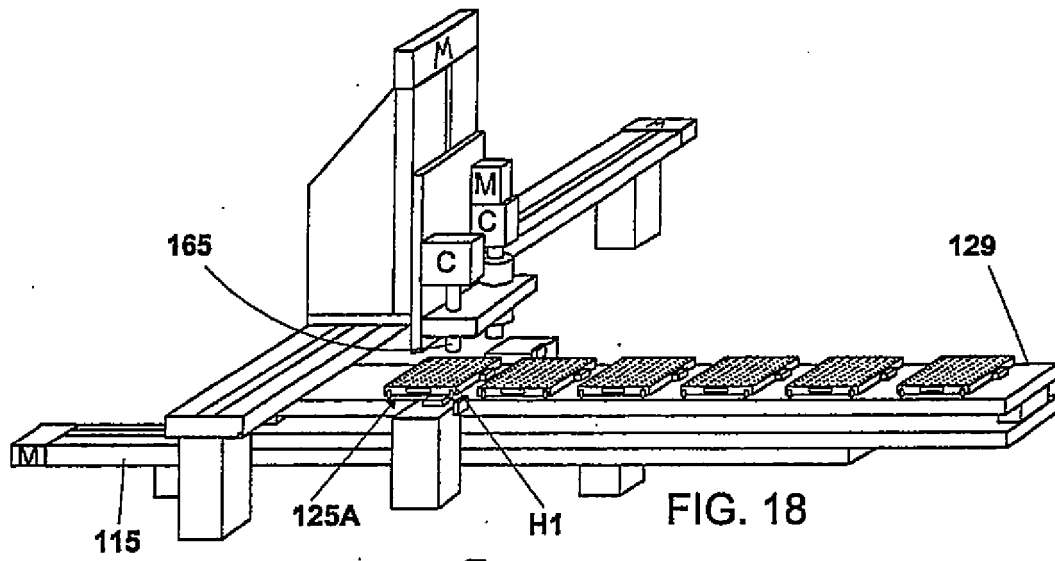
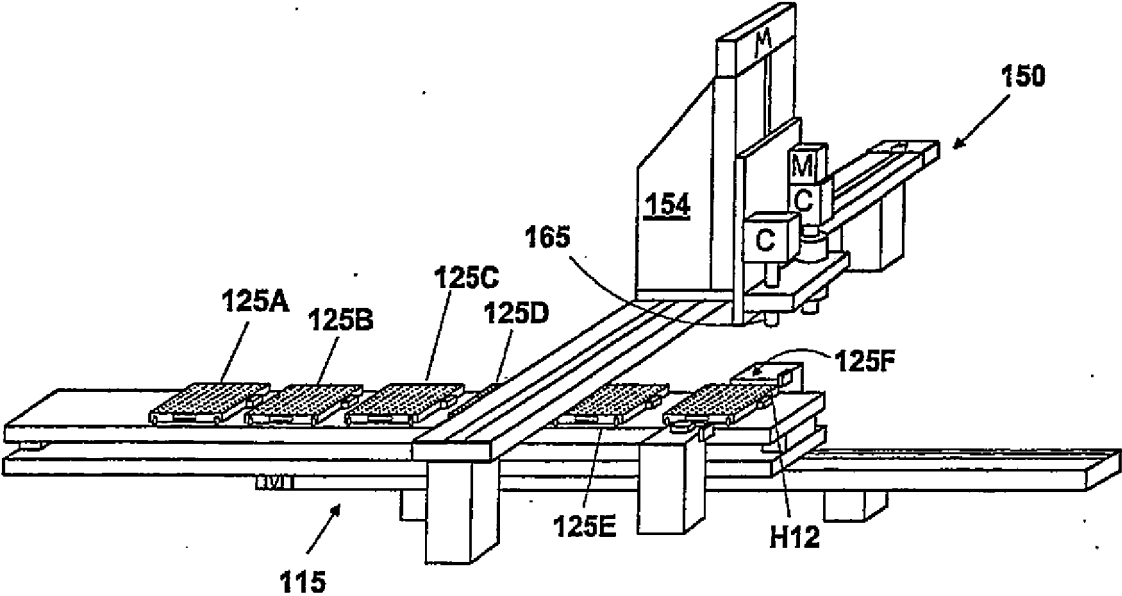
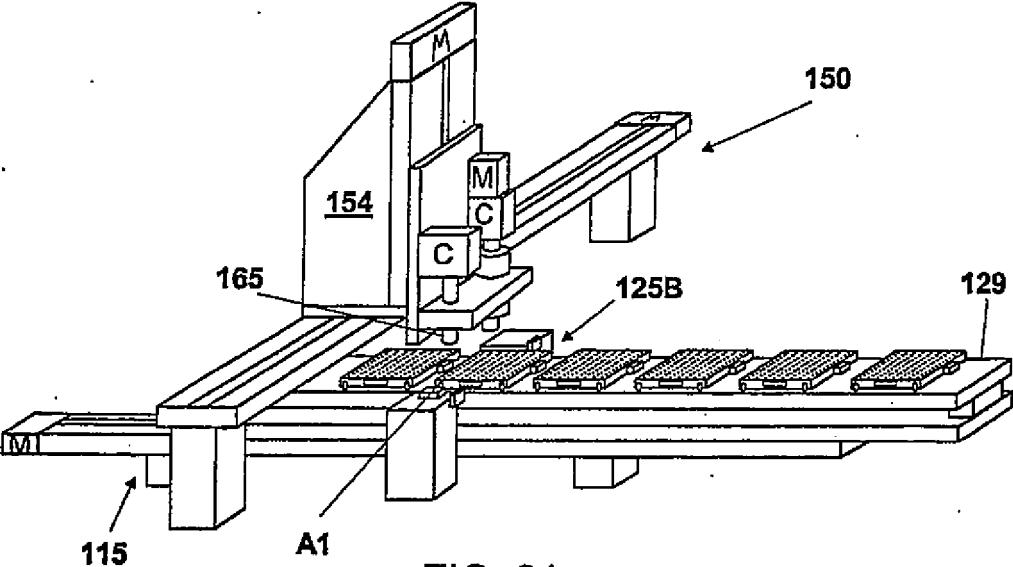
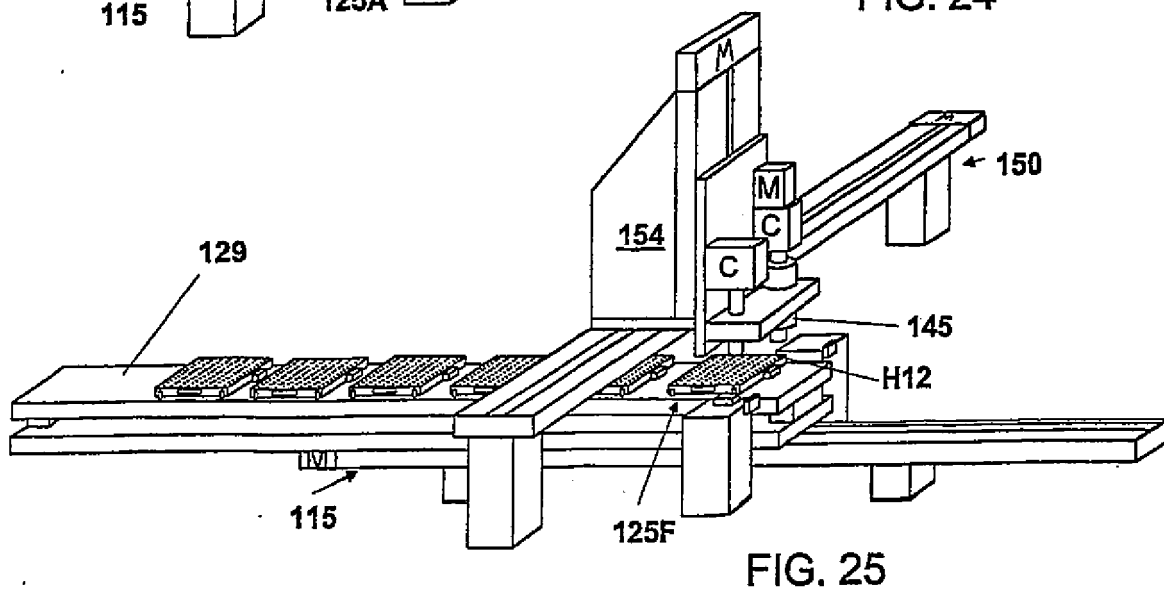
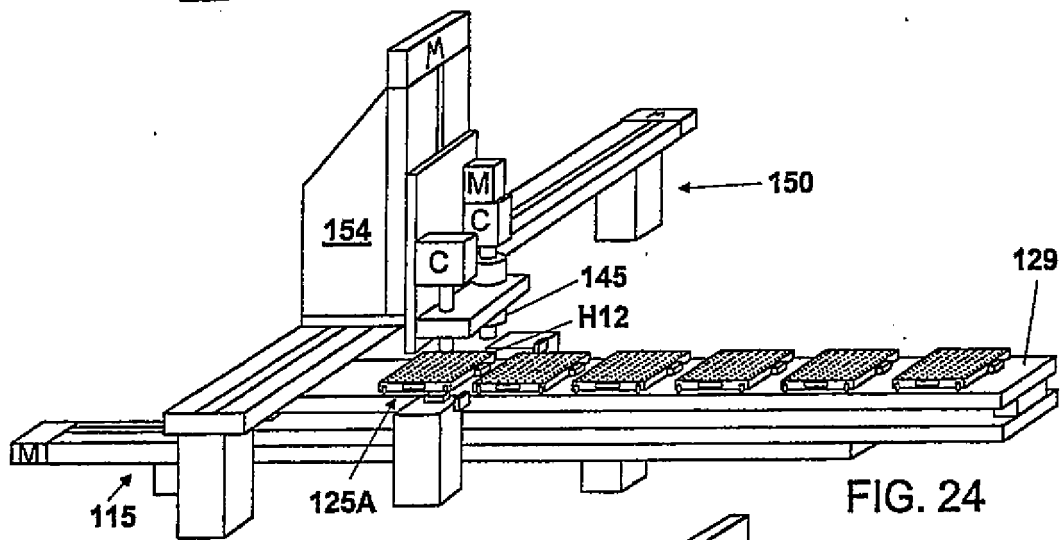
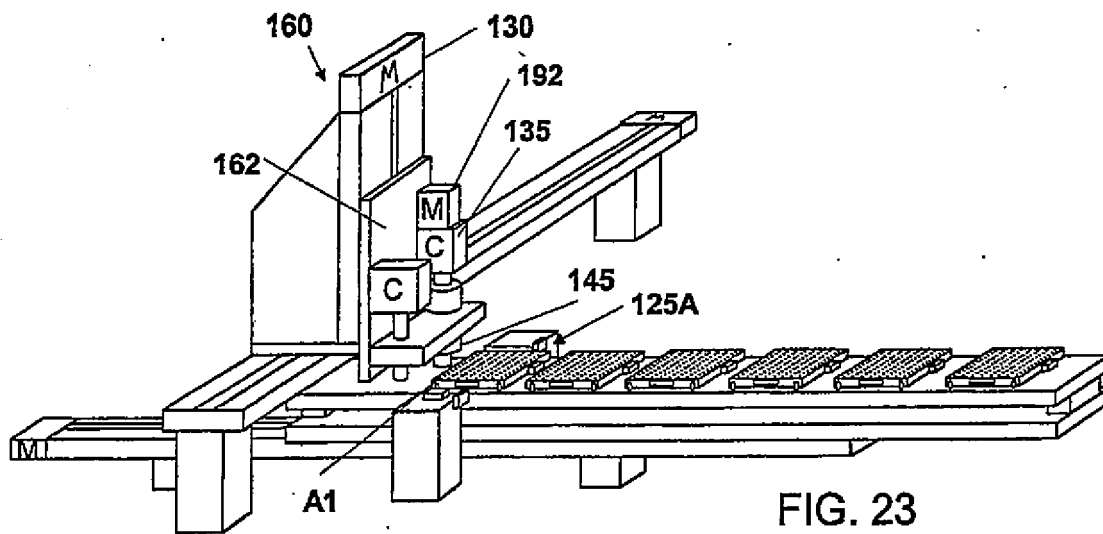


FIG. 17









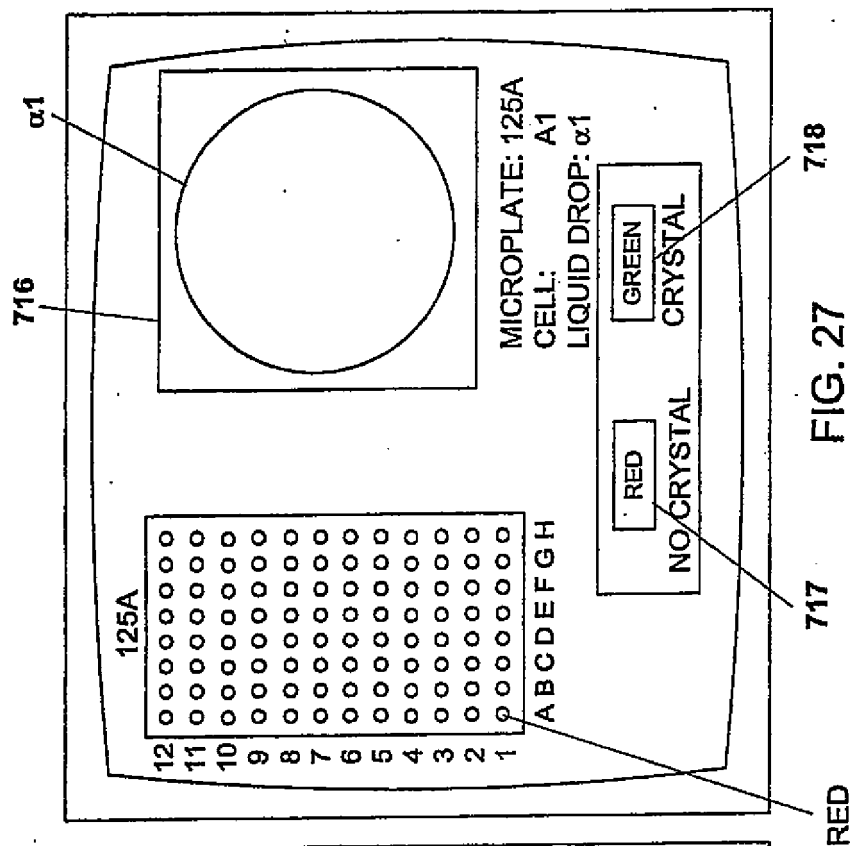


FIG. 26

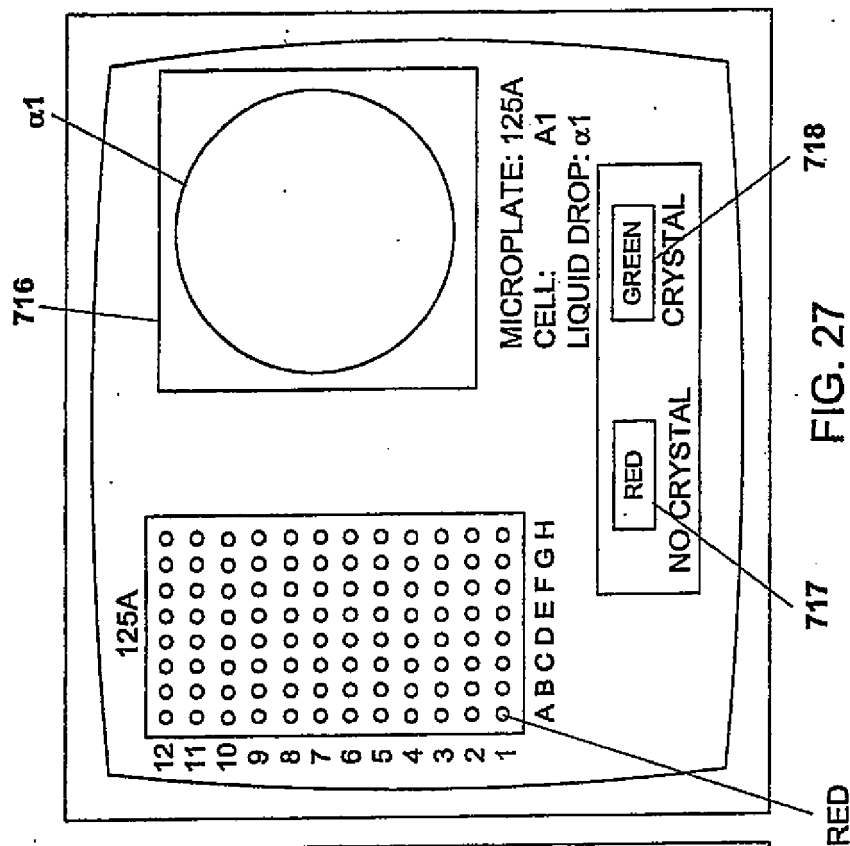


FIG. 27

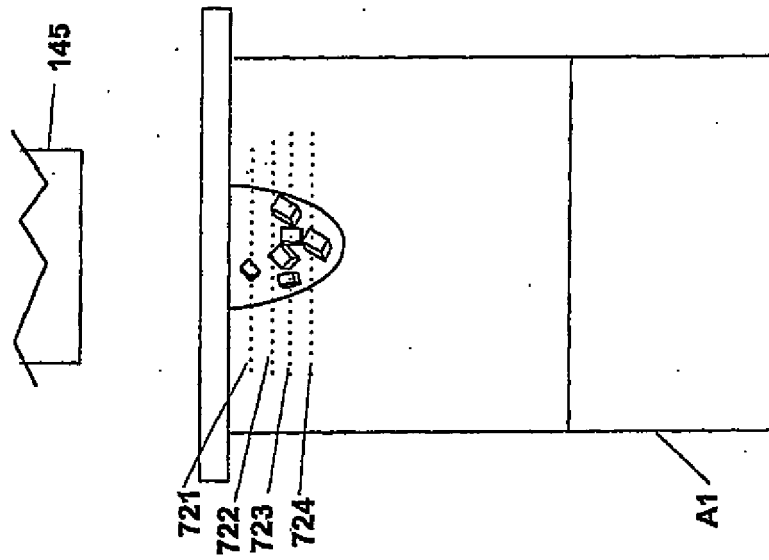


FIG. 29

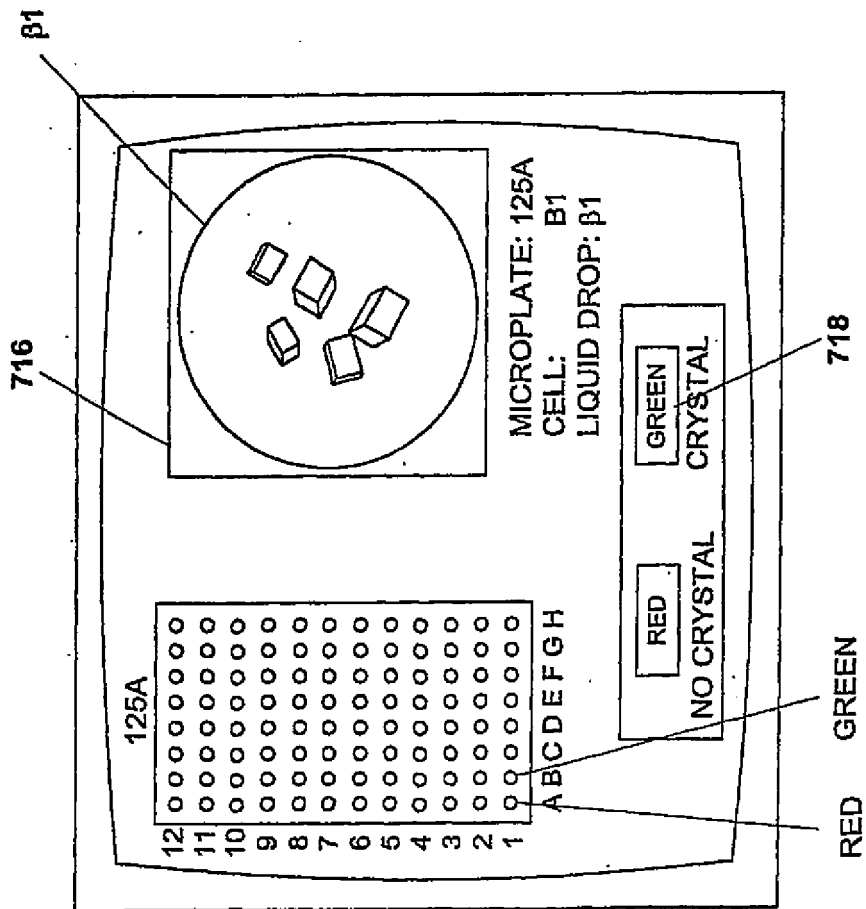


FIG. 28

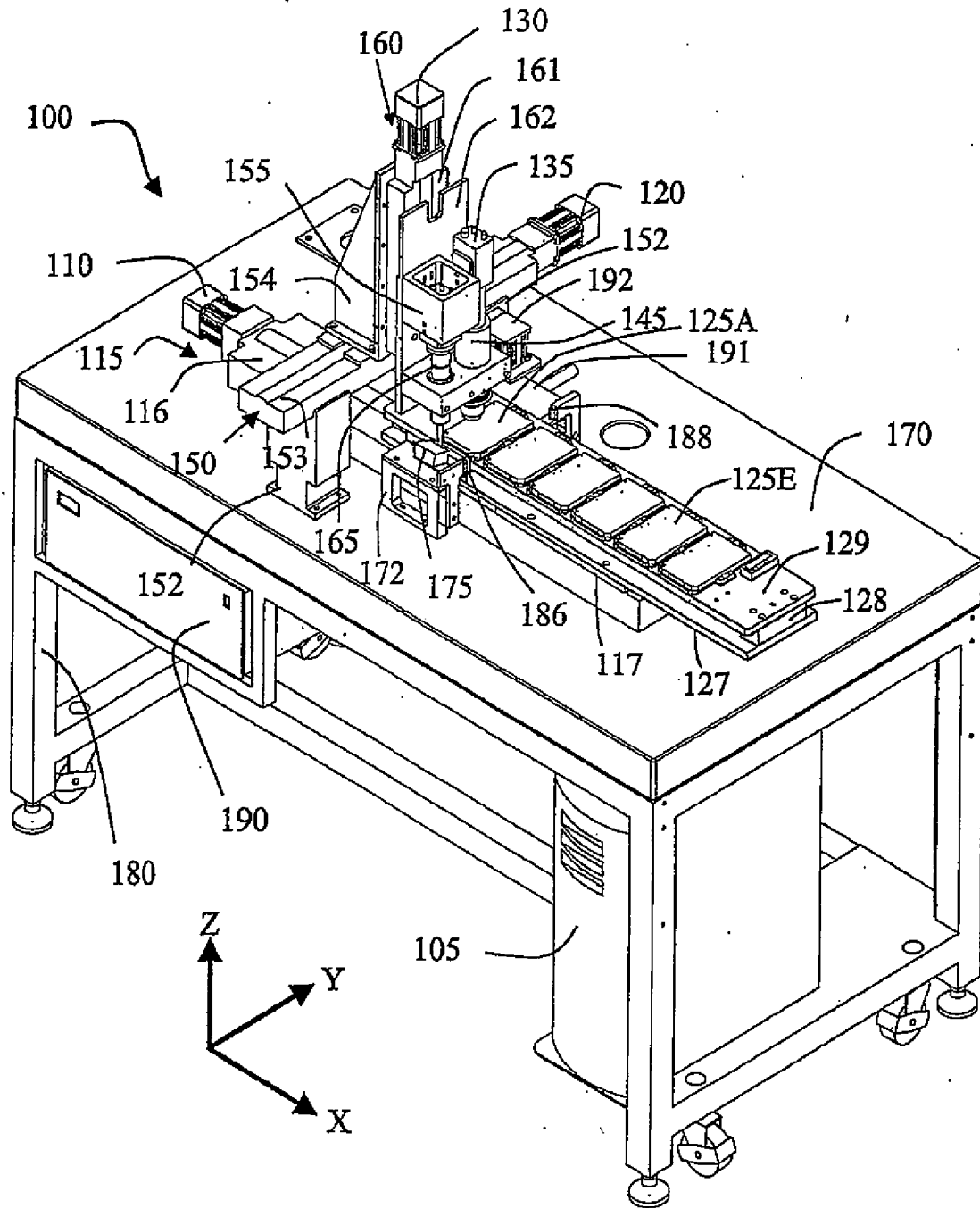


FIG. 30

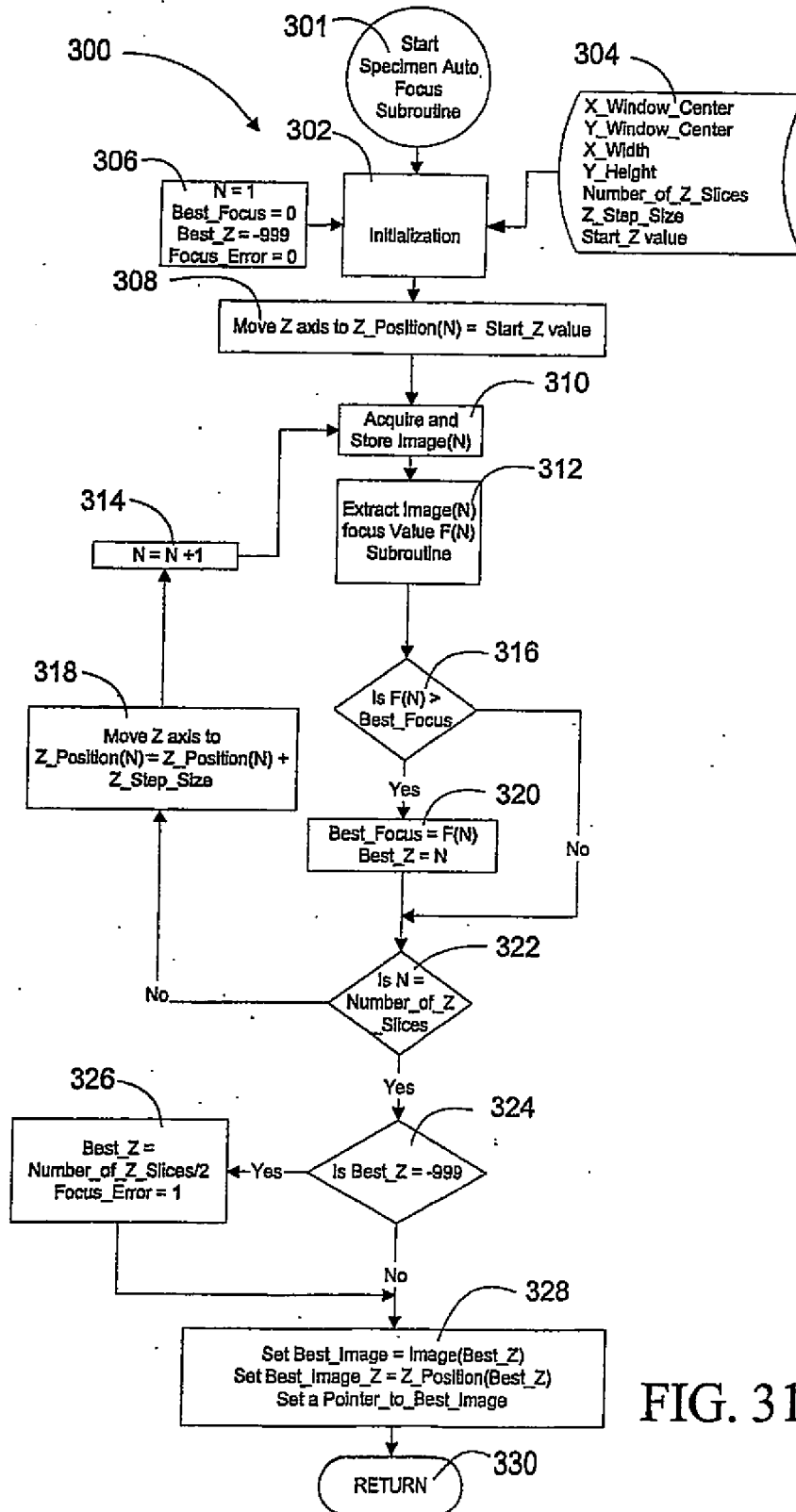


FIG. 31

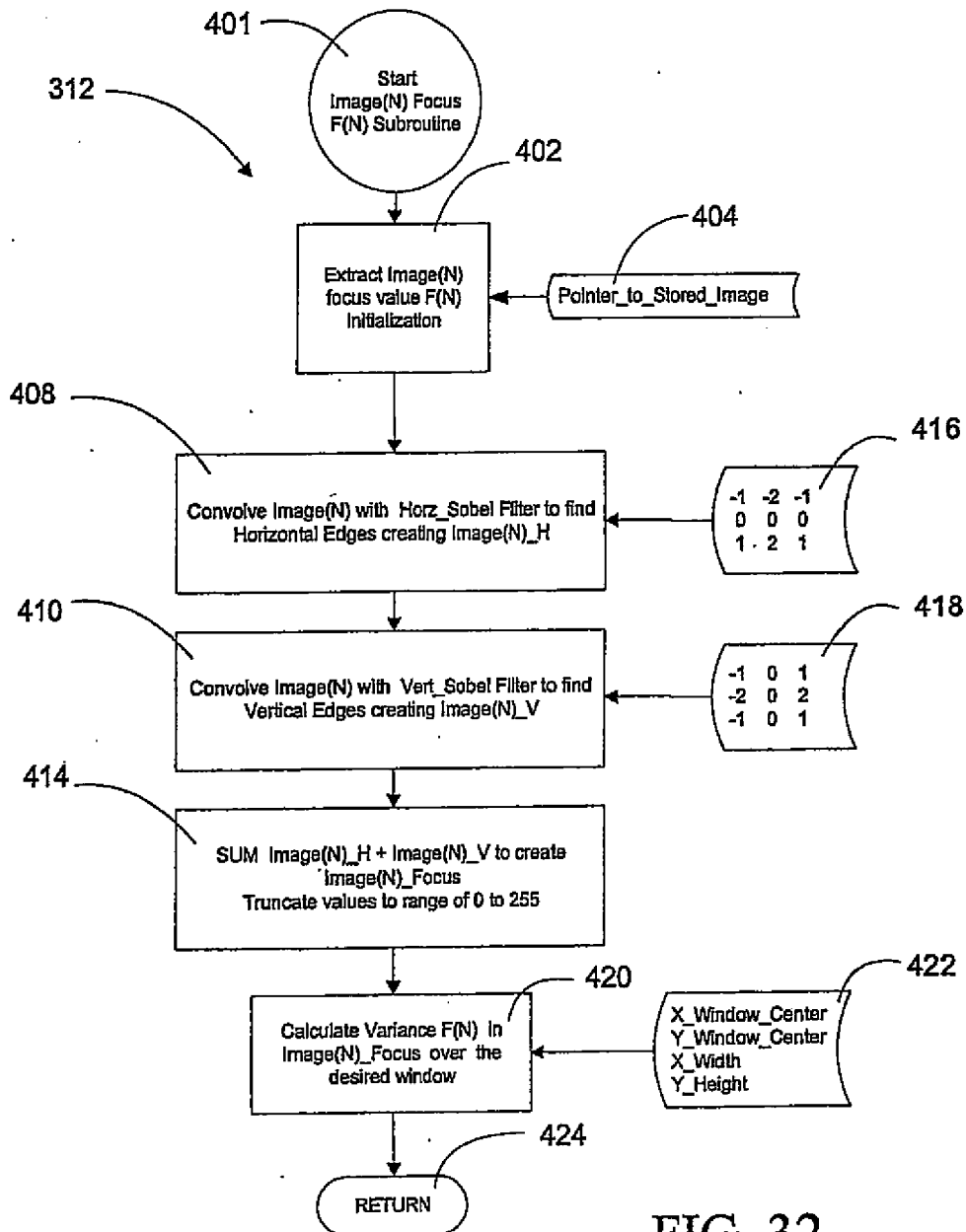


FIG. 32

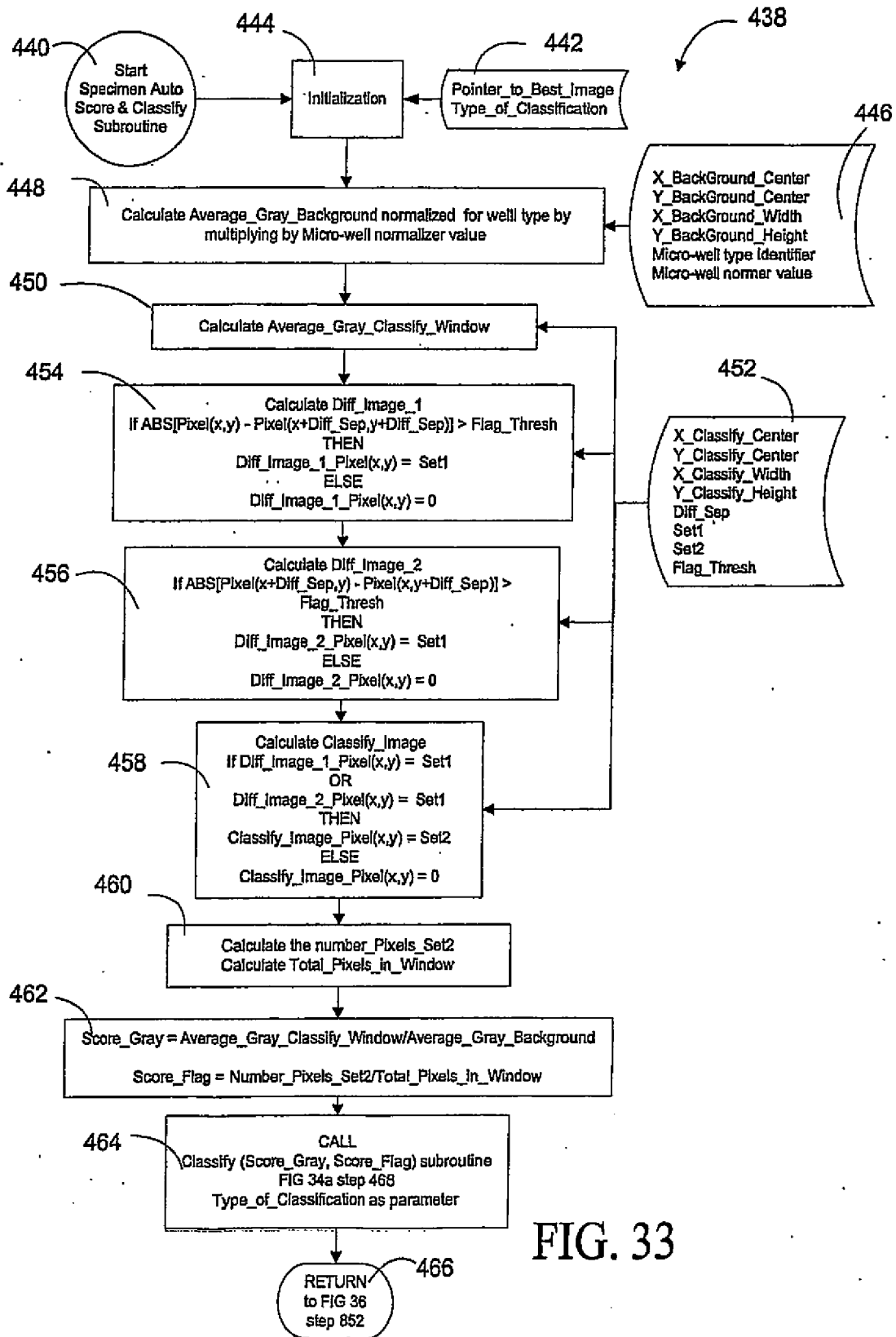


FIG. 33

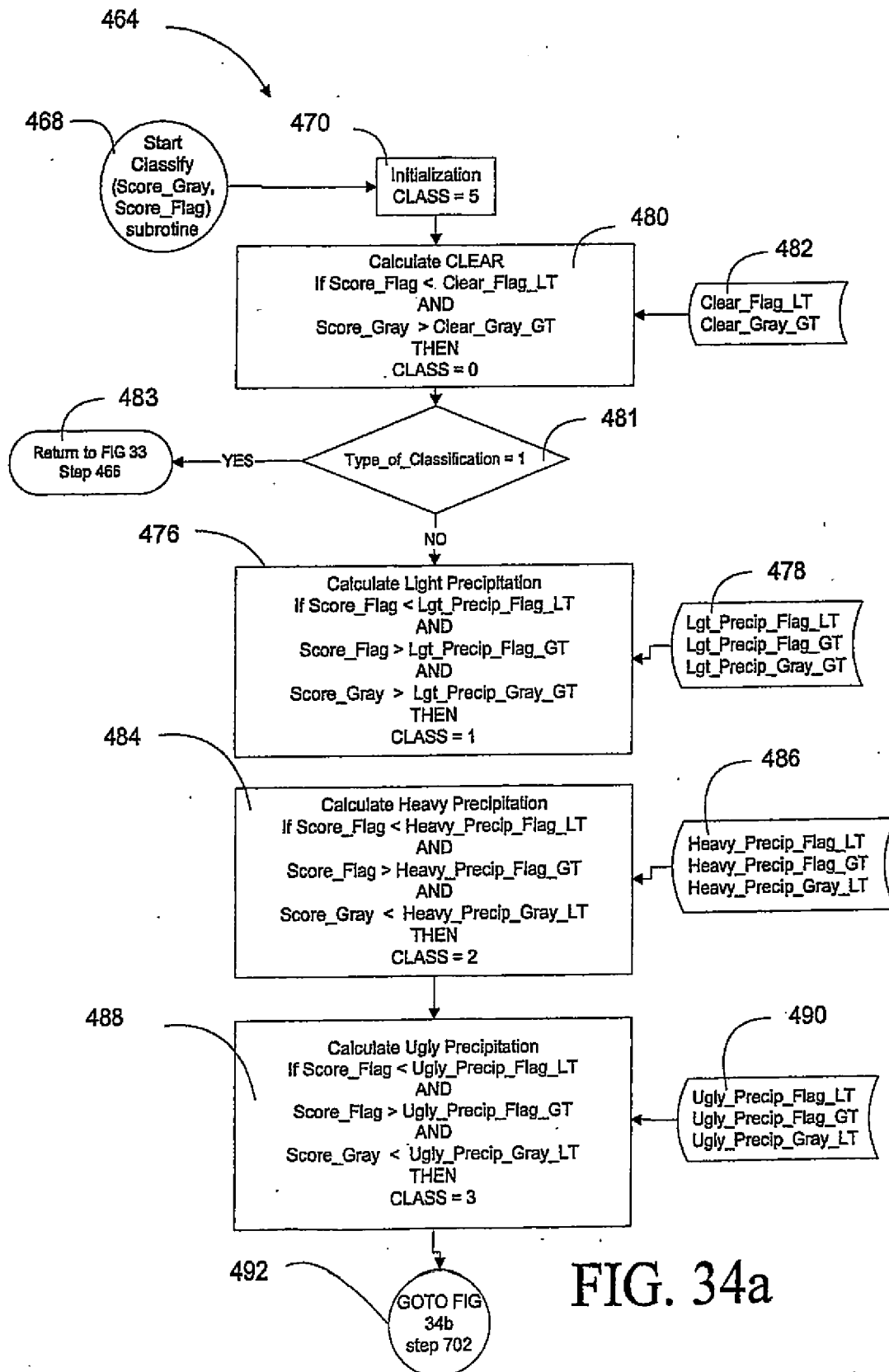


FIG. 34a



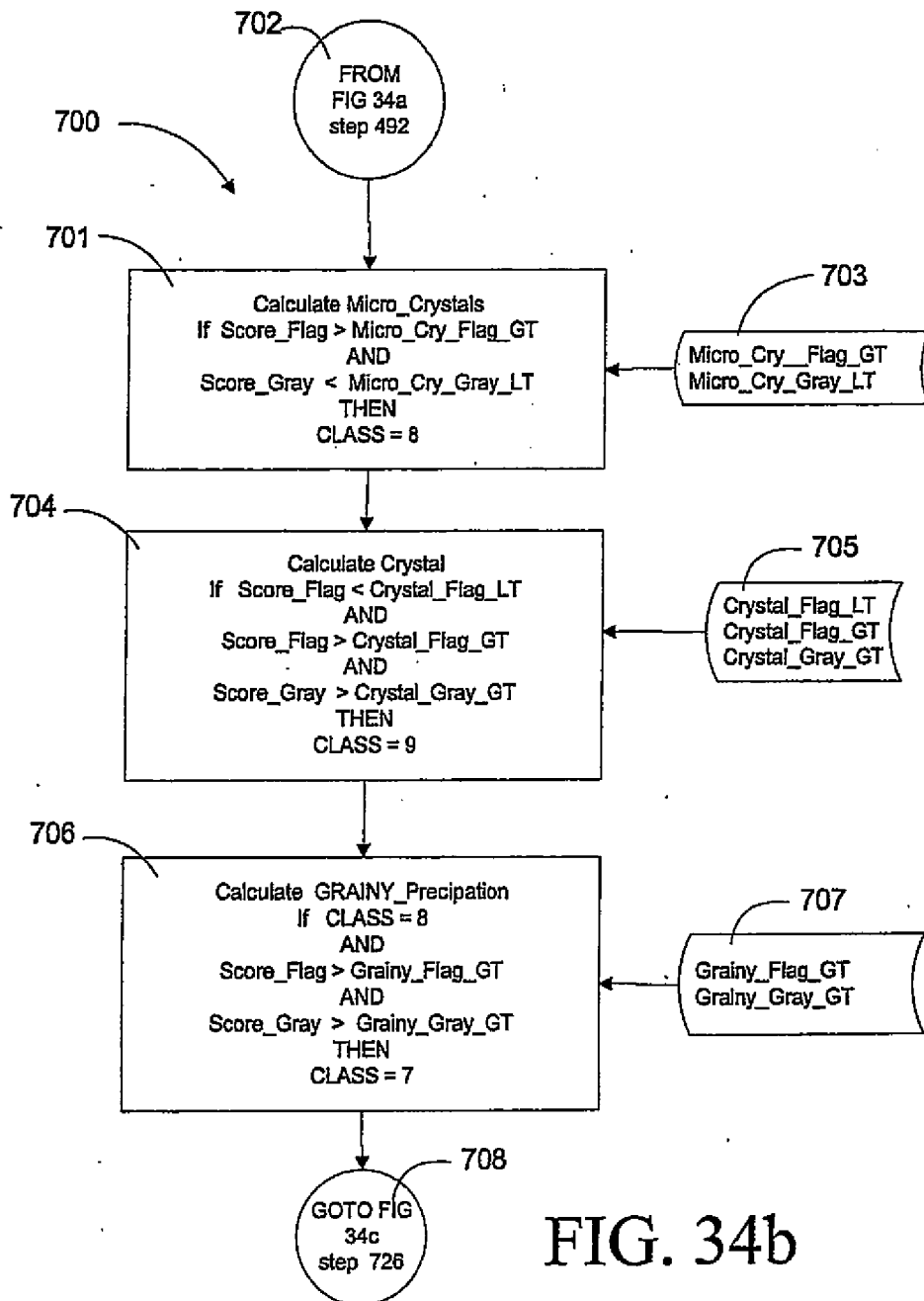


FIG. 34b

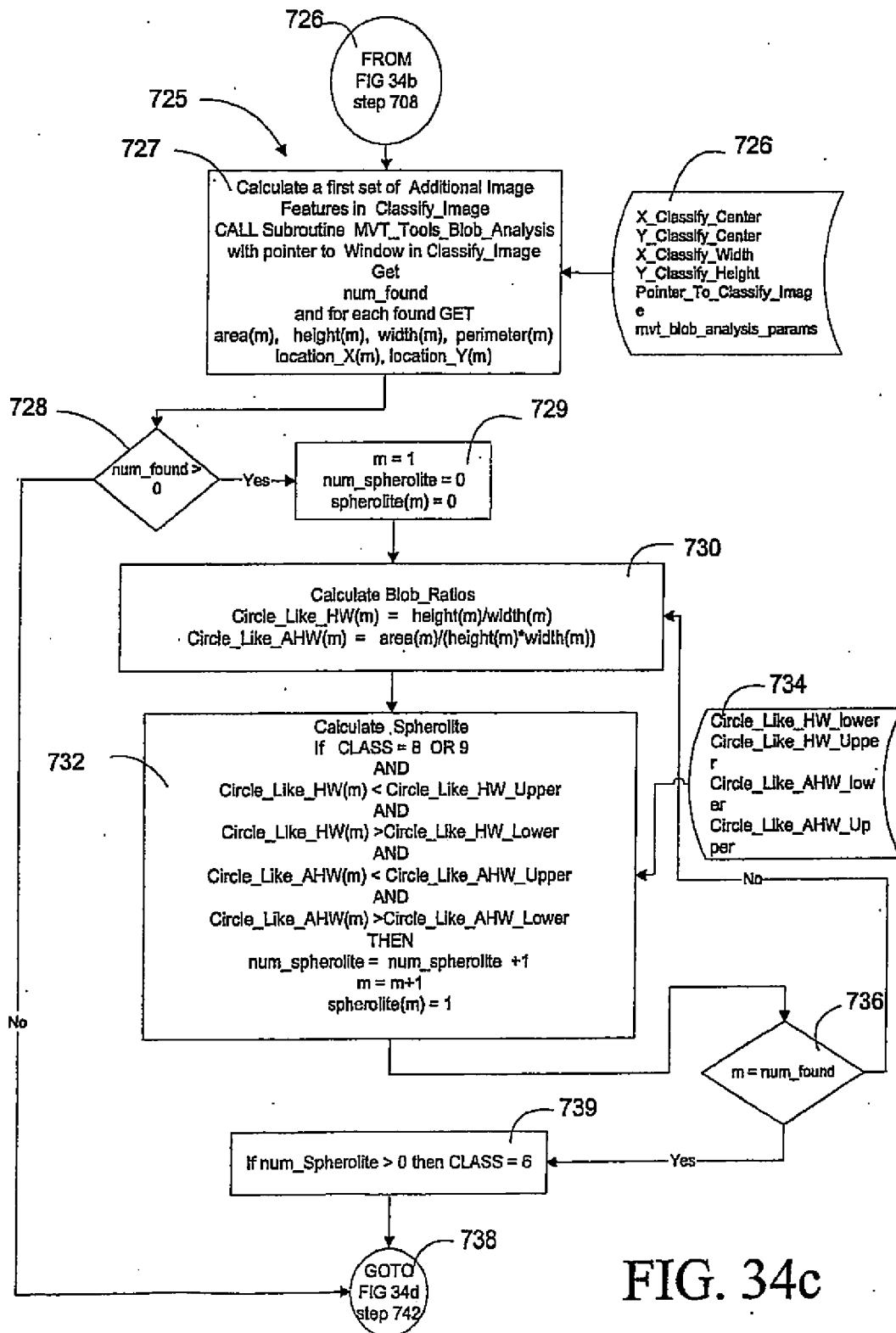


FIG. 34c

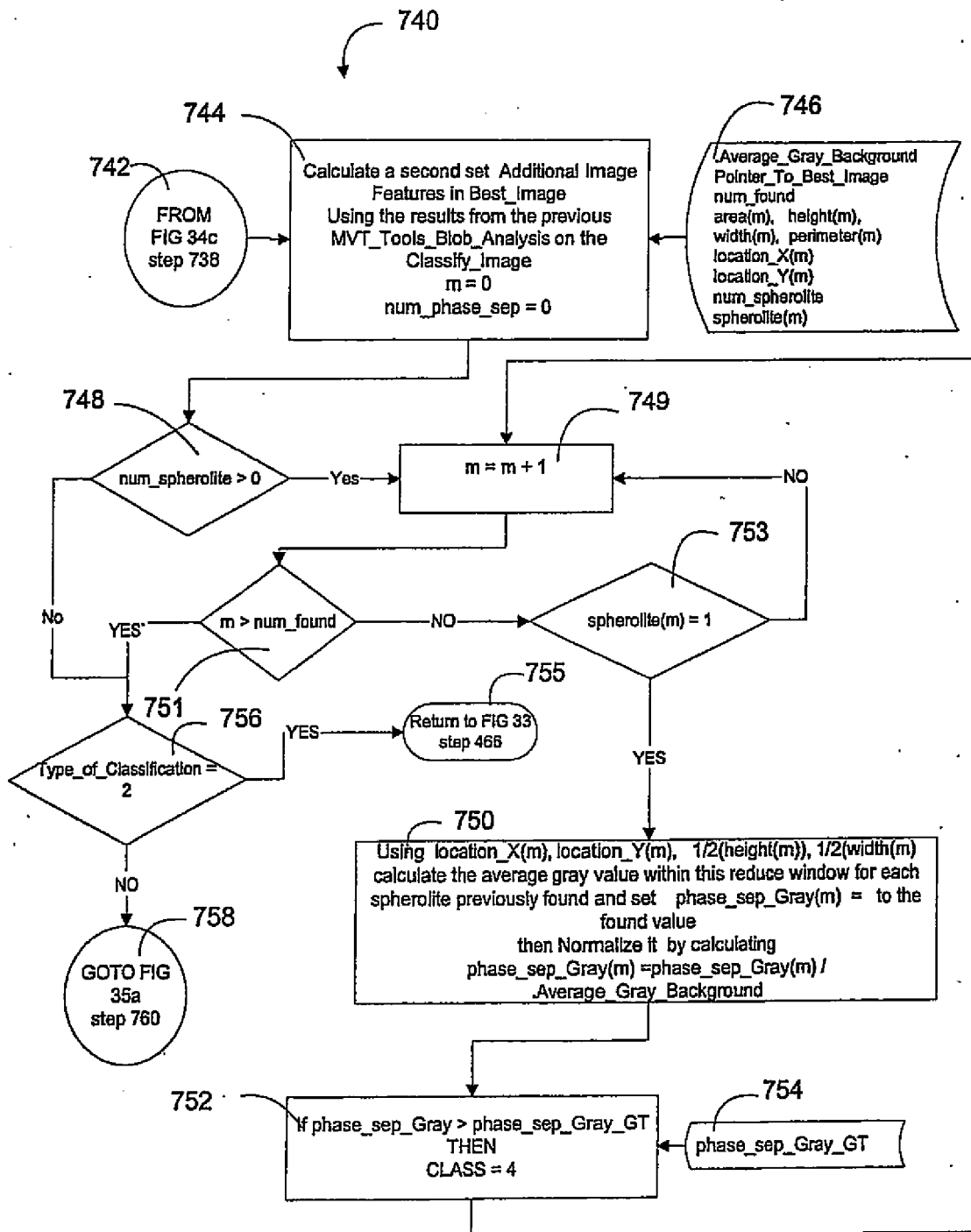


FIG. 34d

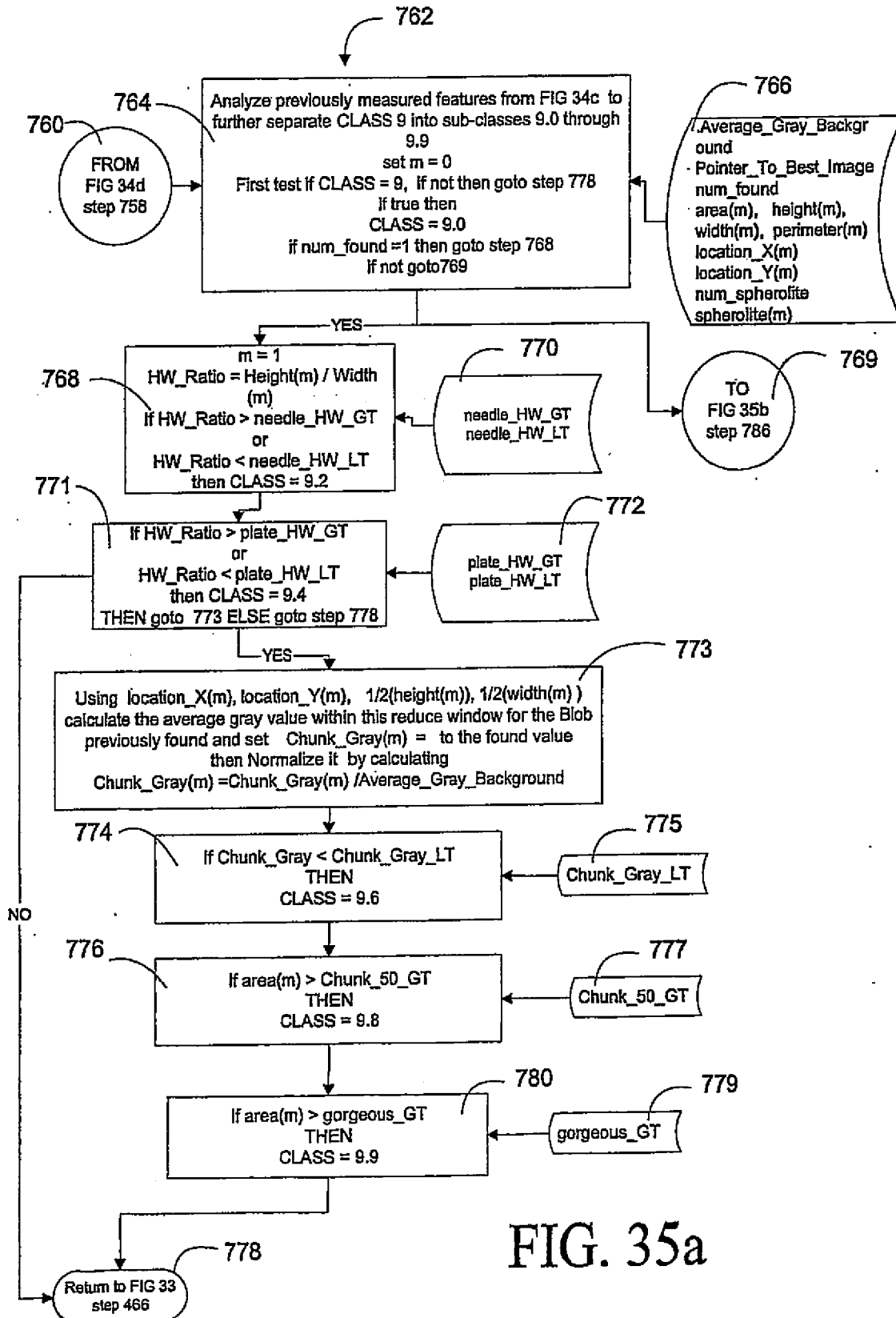


FIG. 35a

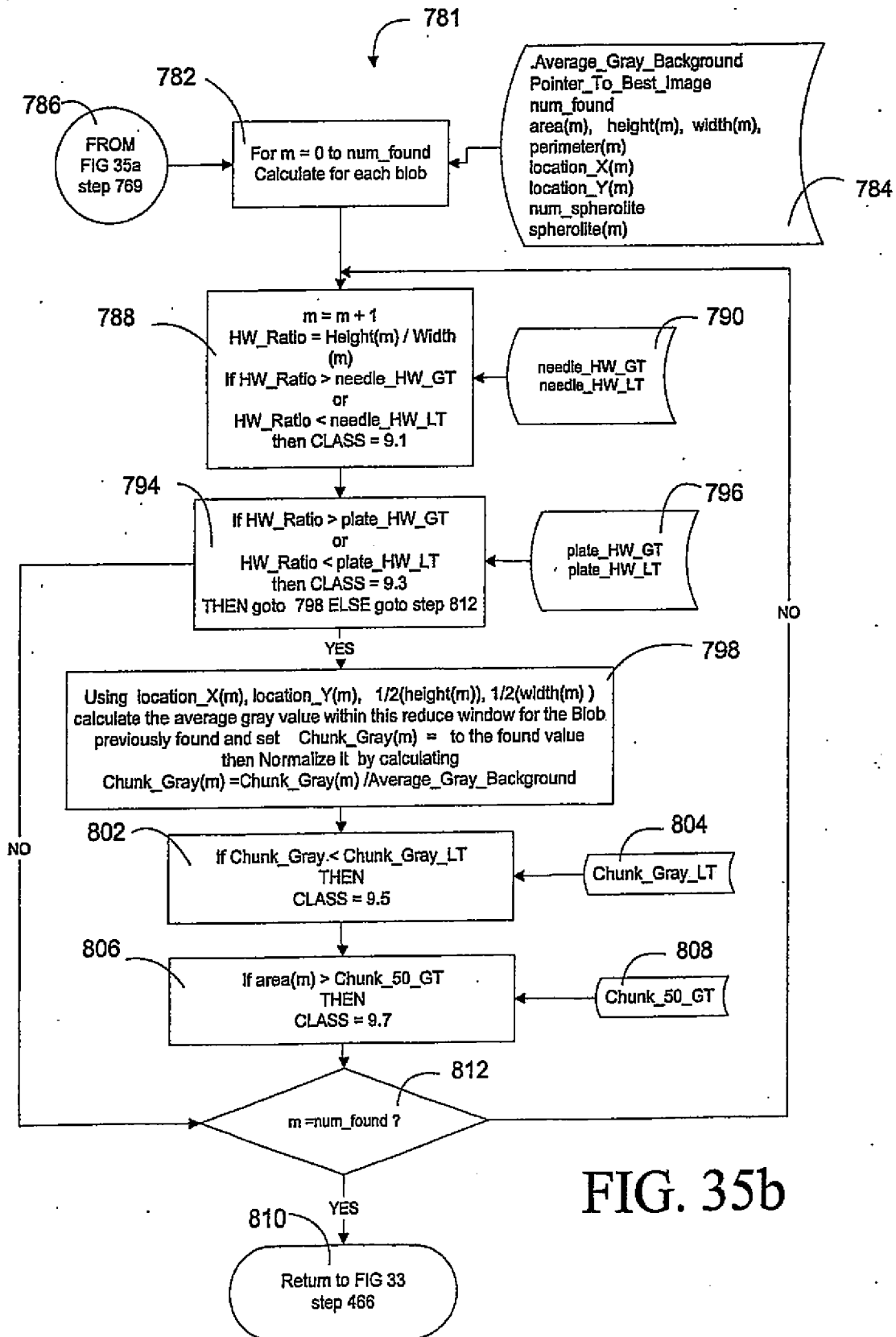
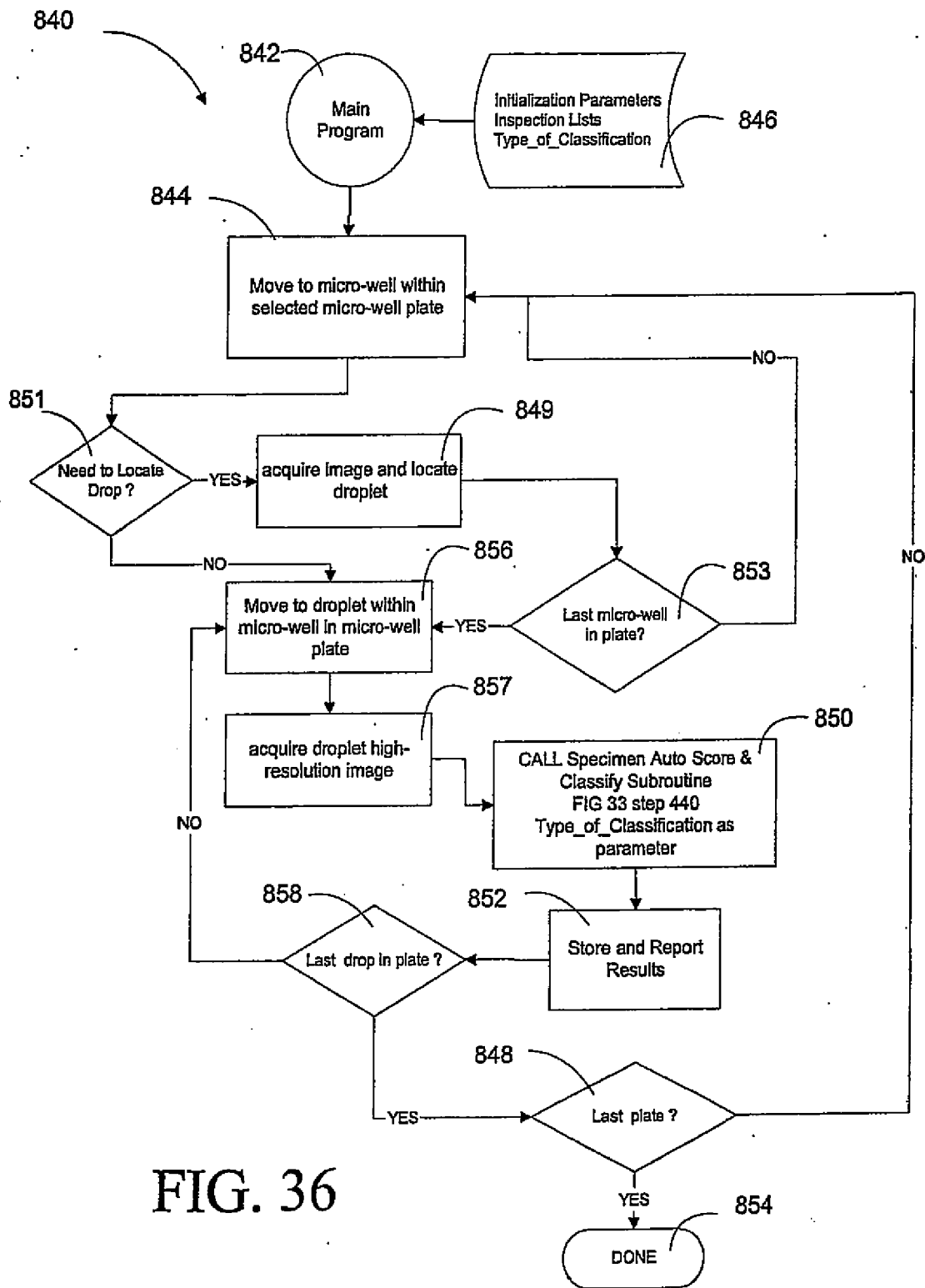


FIG. 35b



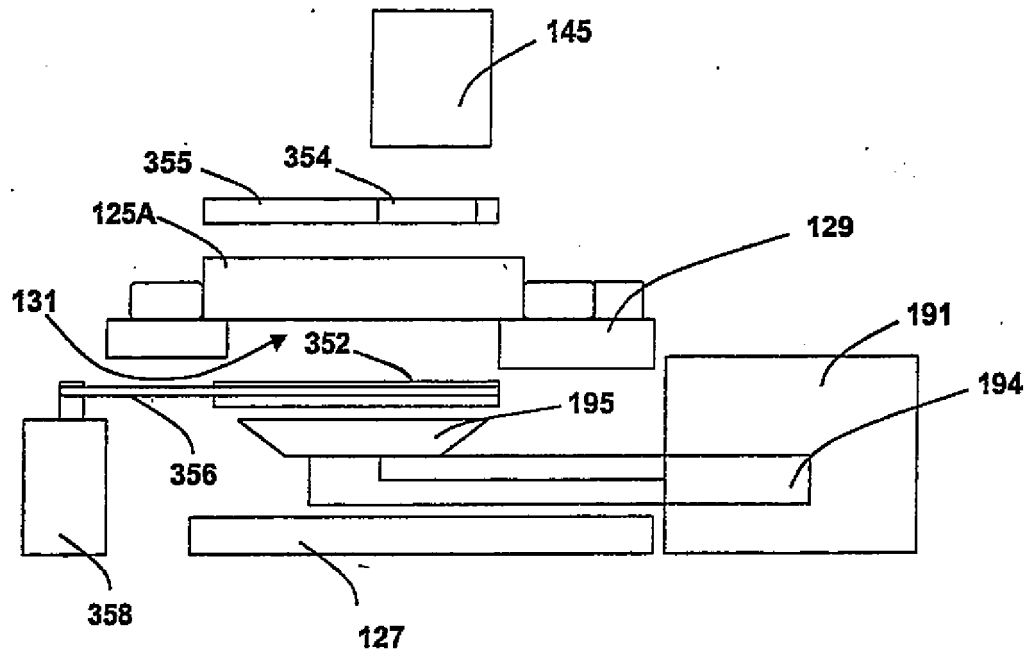


FIG. 37

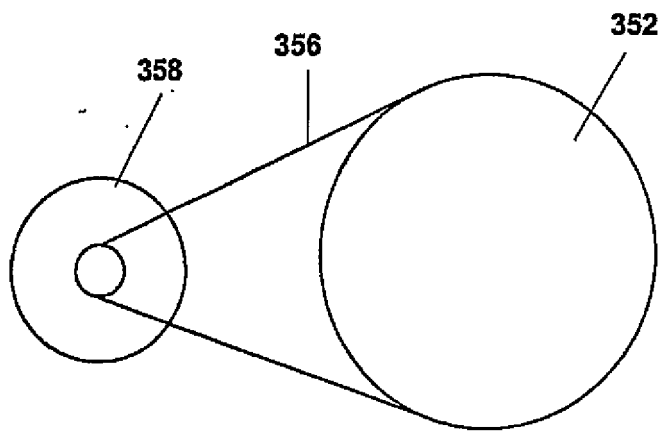


FIG. 38

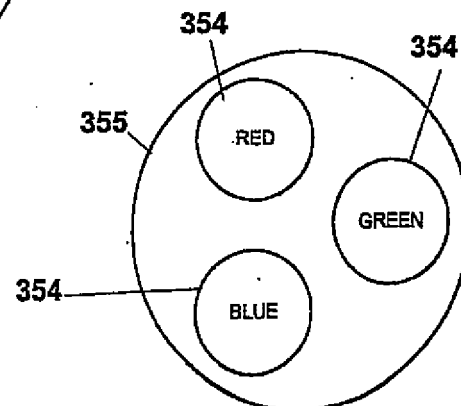


FIG. 39

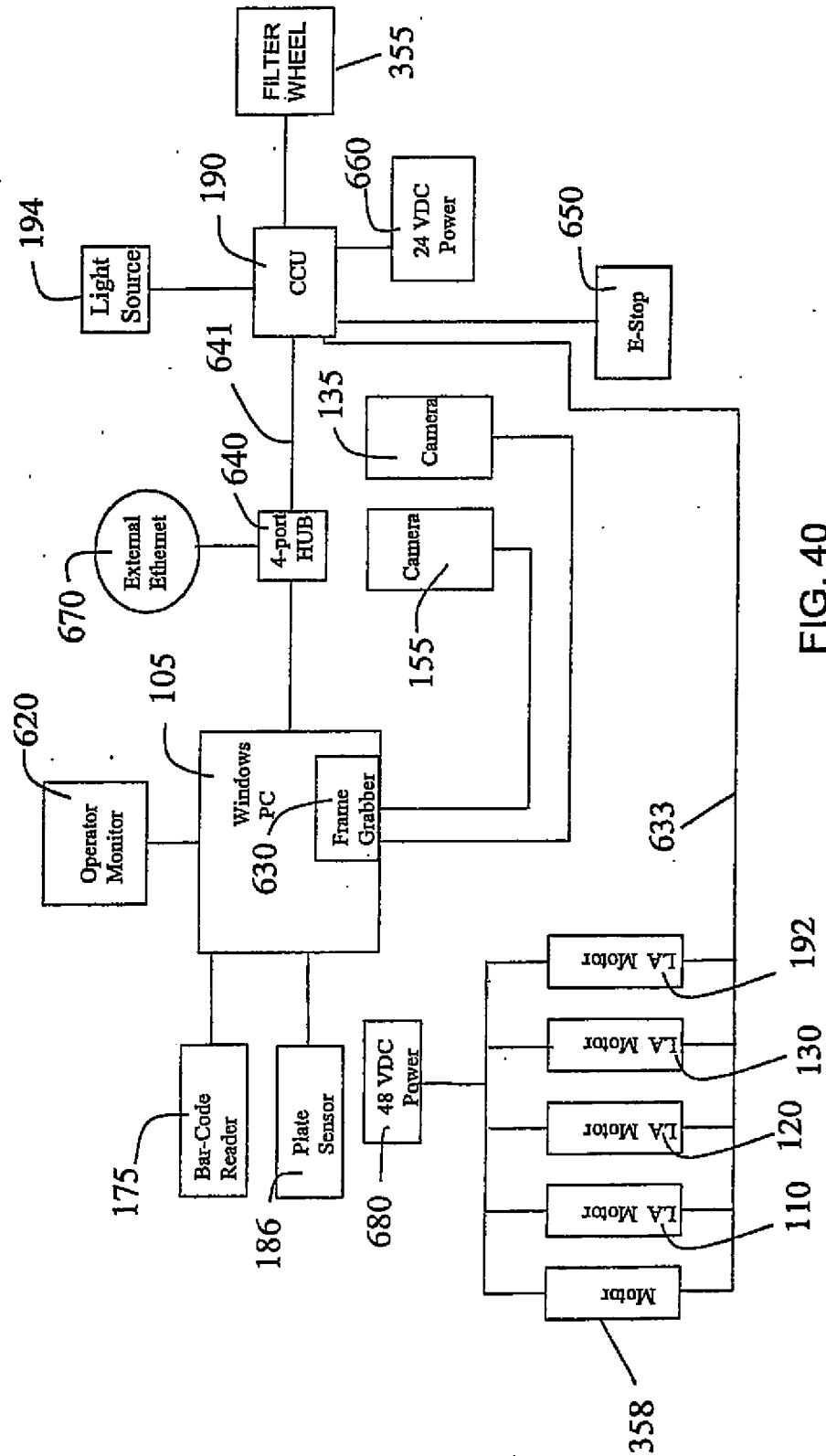


FIG. 40



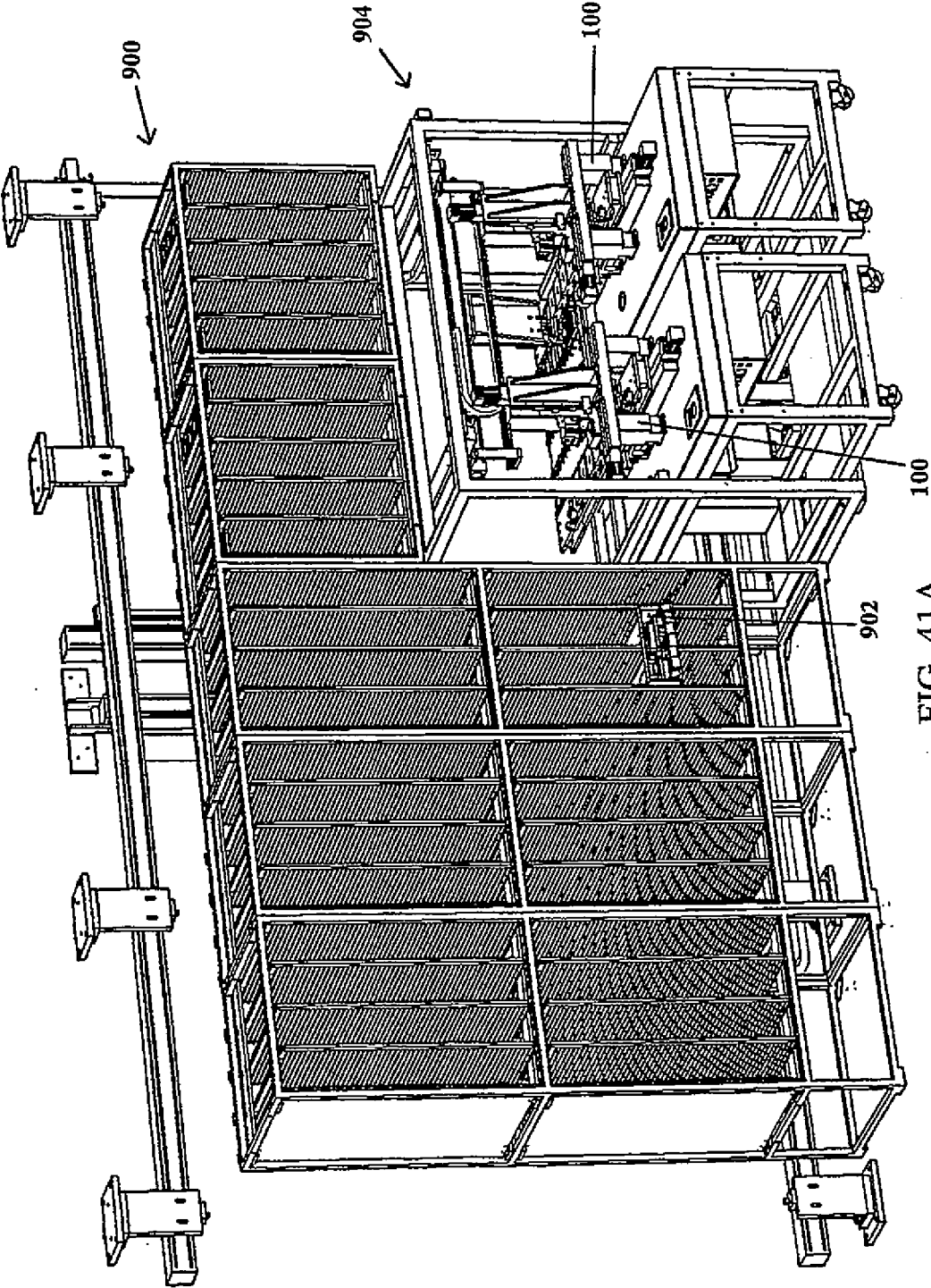


FIG. 41A

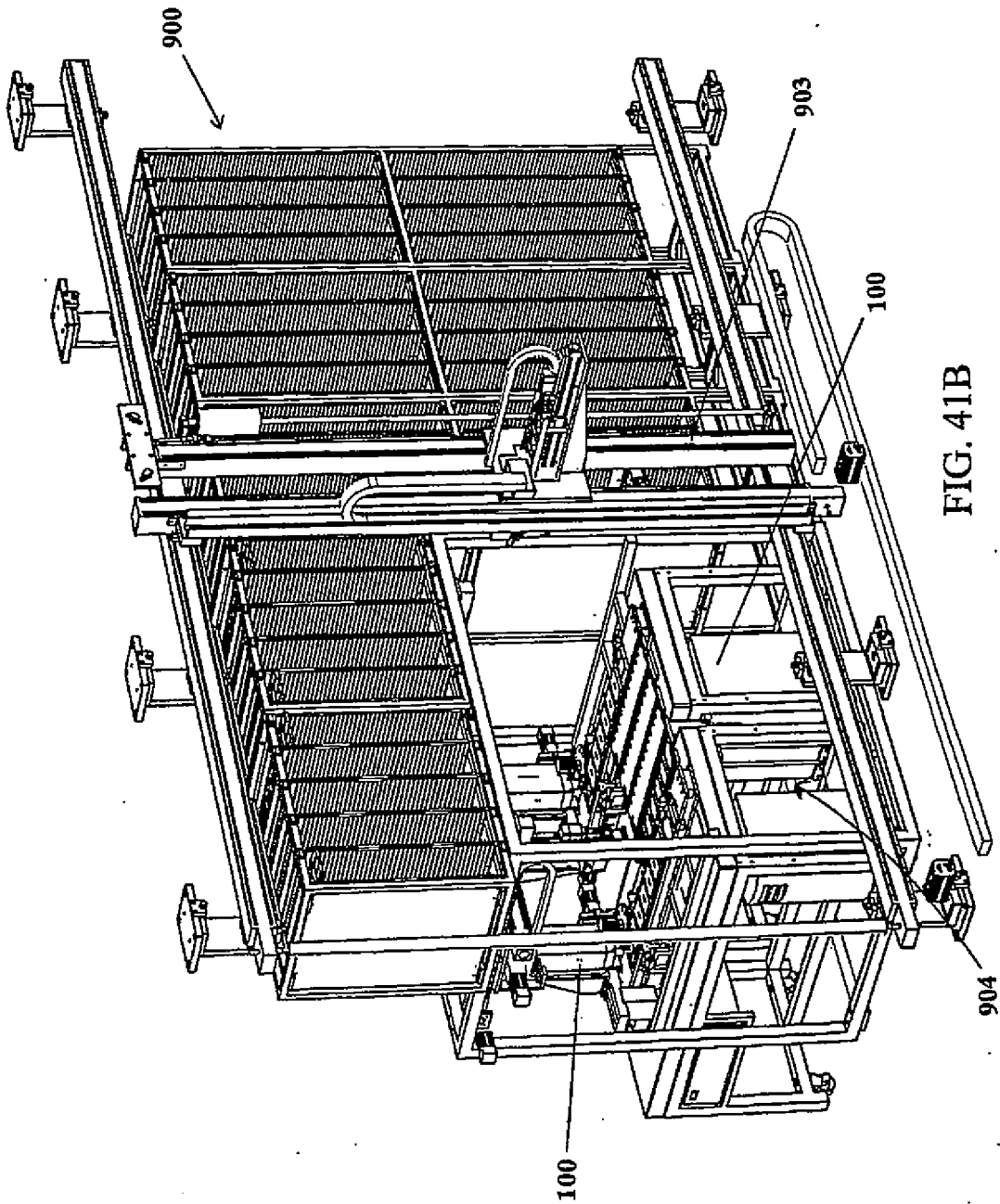


FIG. 41B

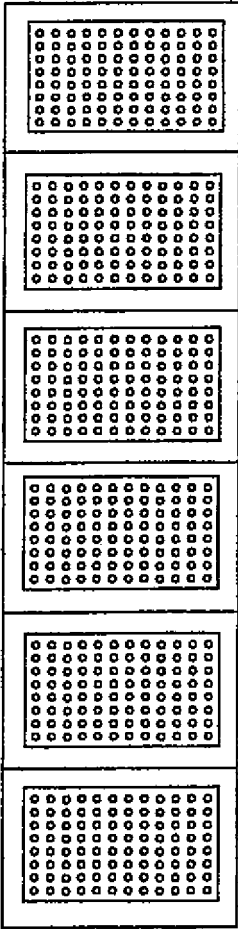


FIG. 42

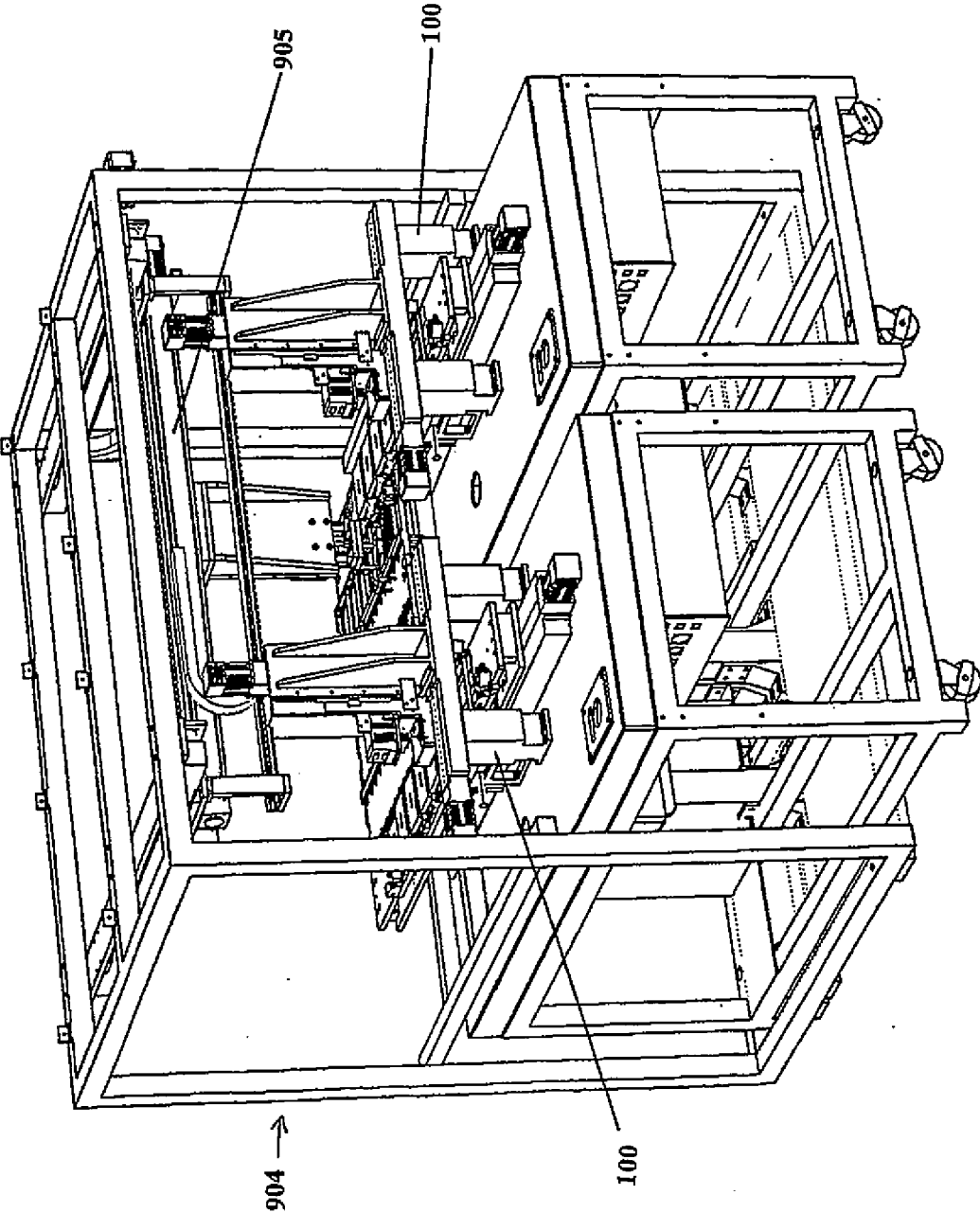


FIG. 43

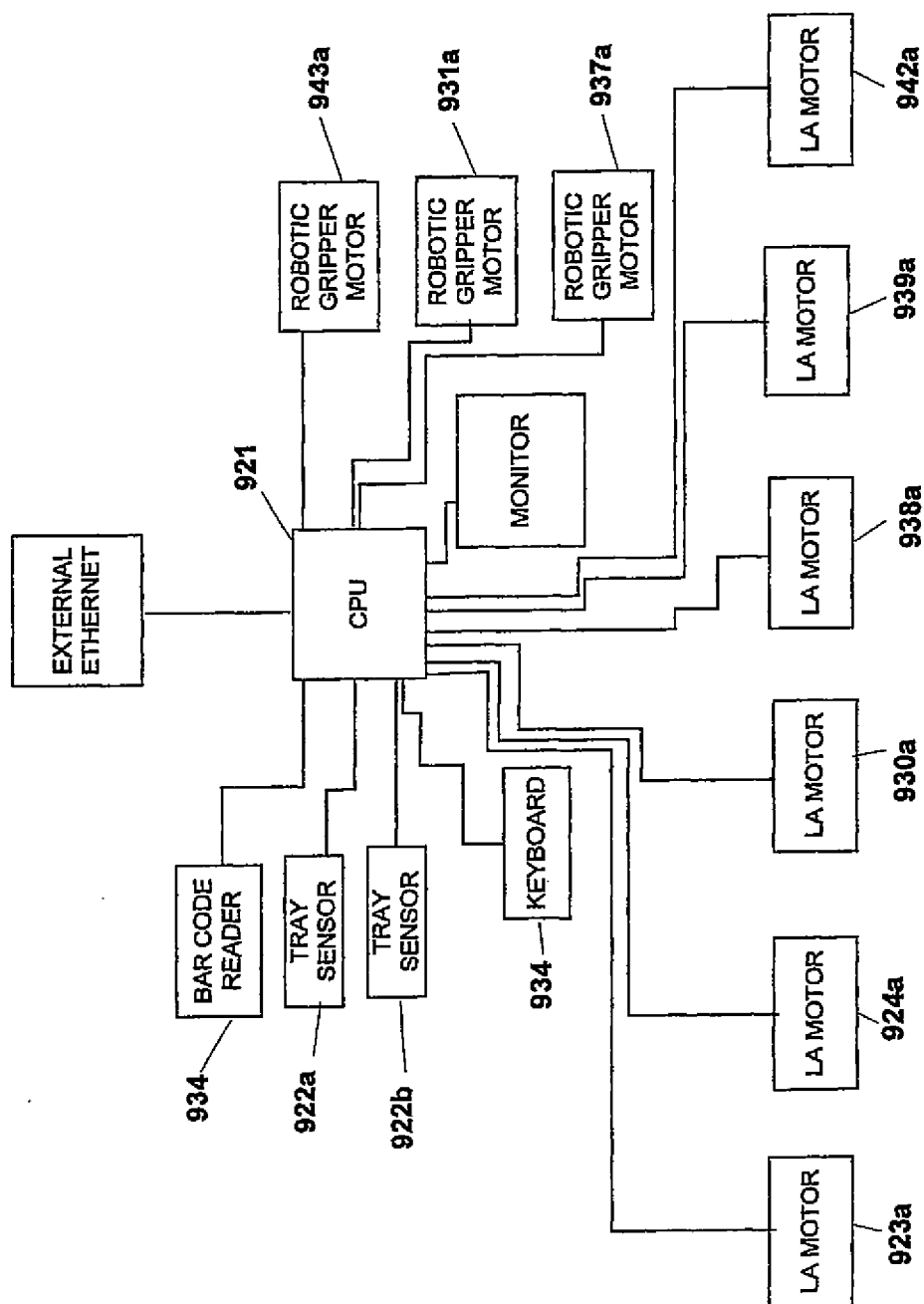


FIG. 44

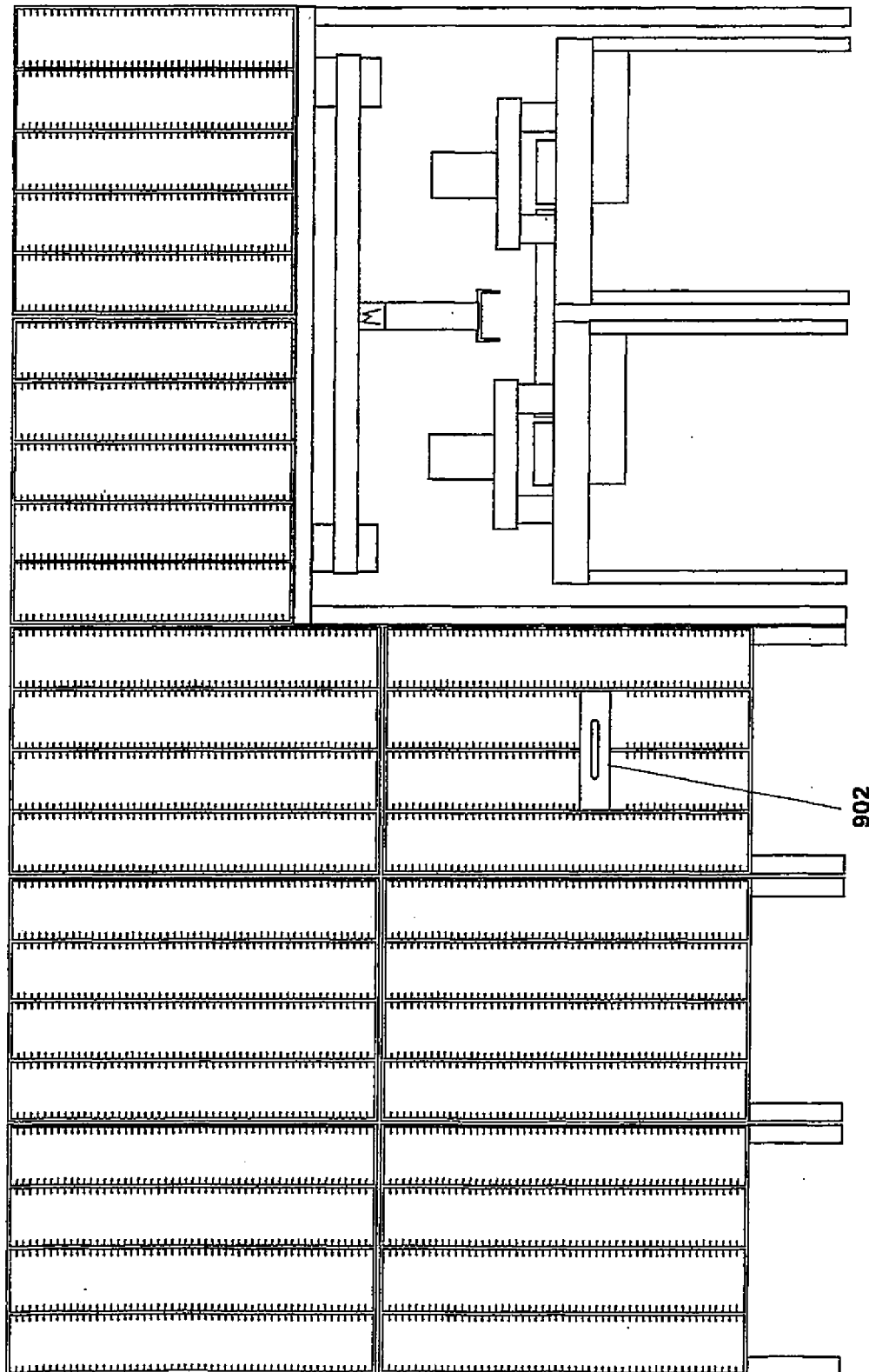
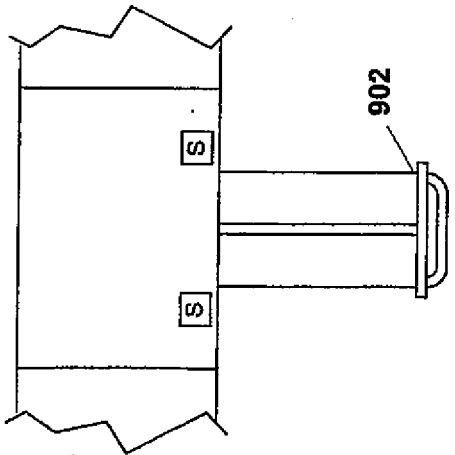
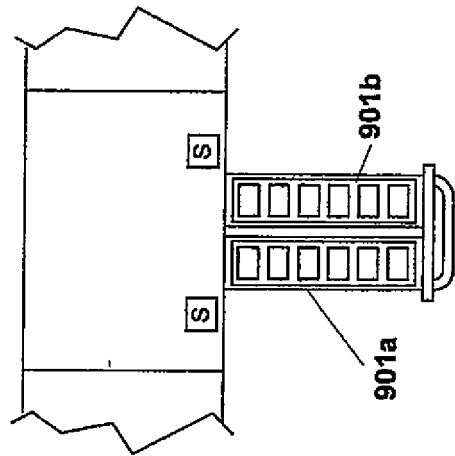
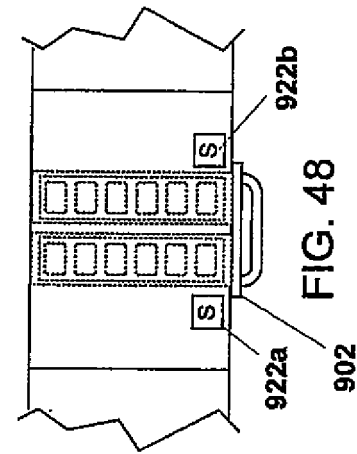
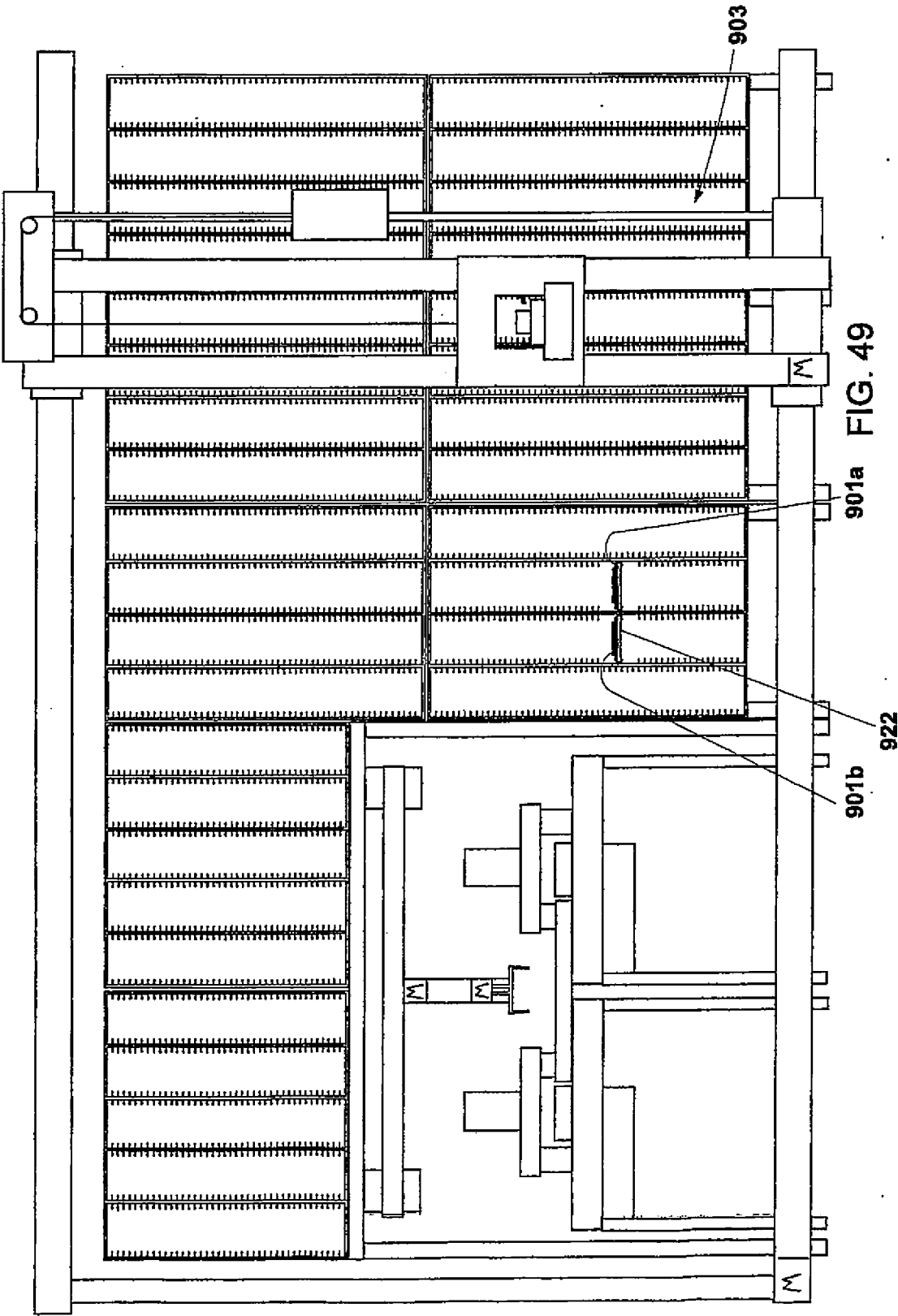
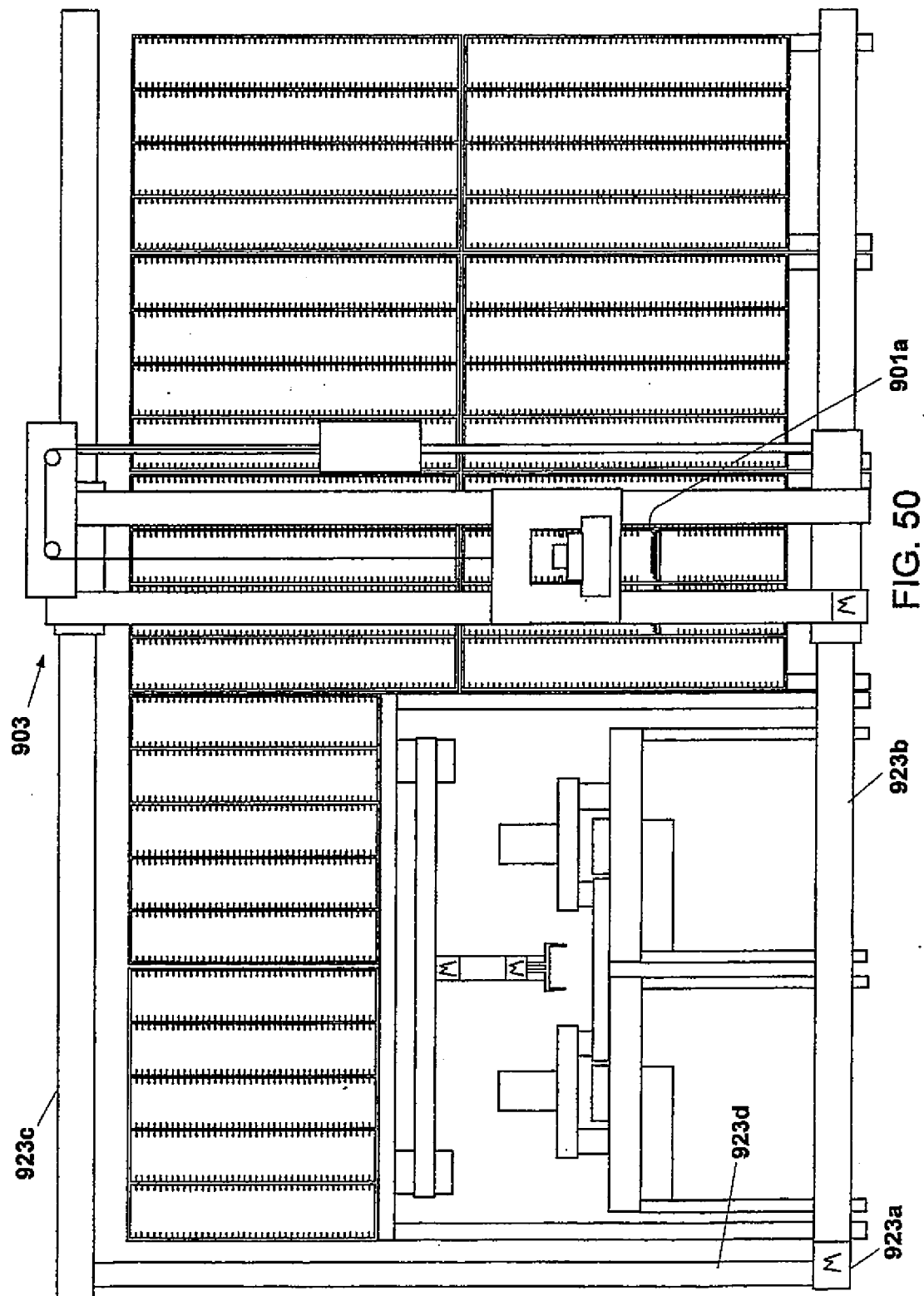


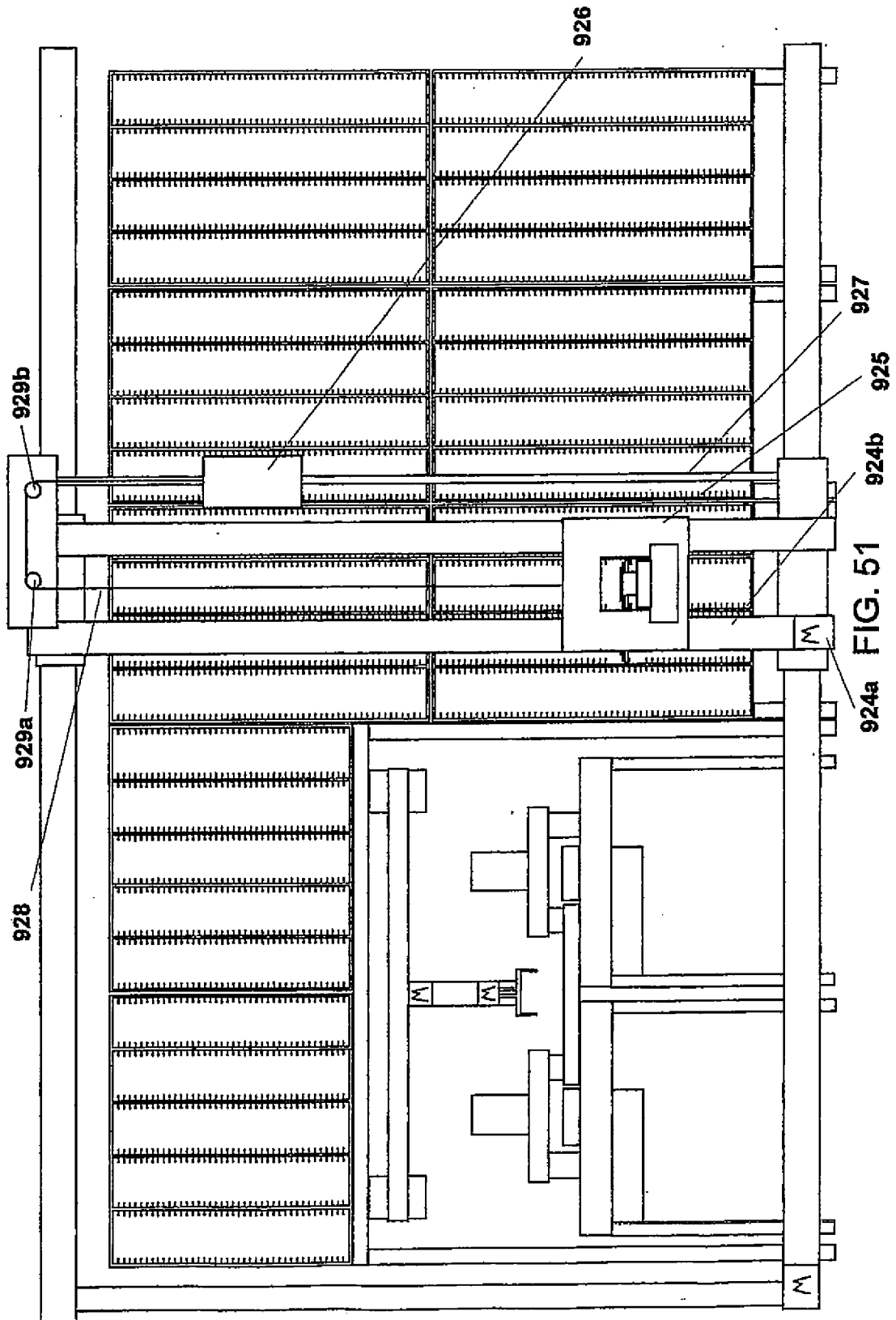
FIG. 45

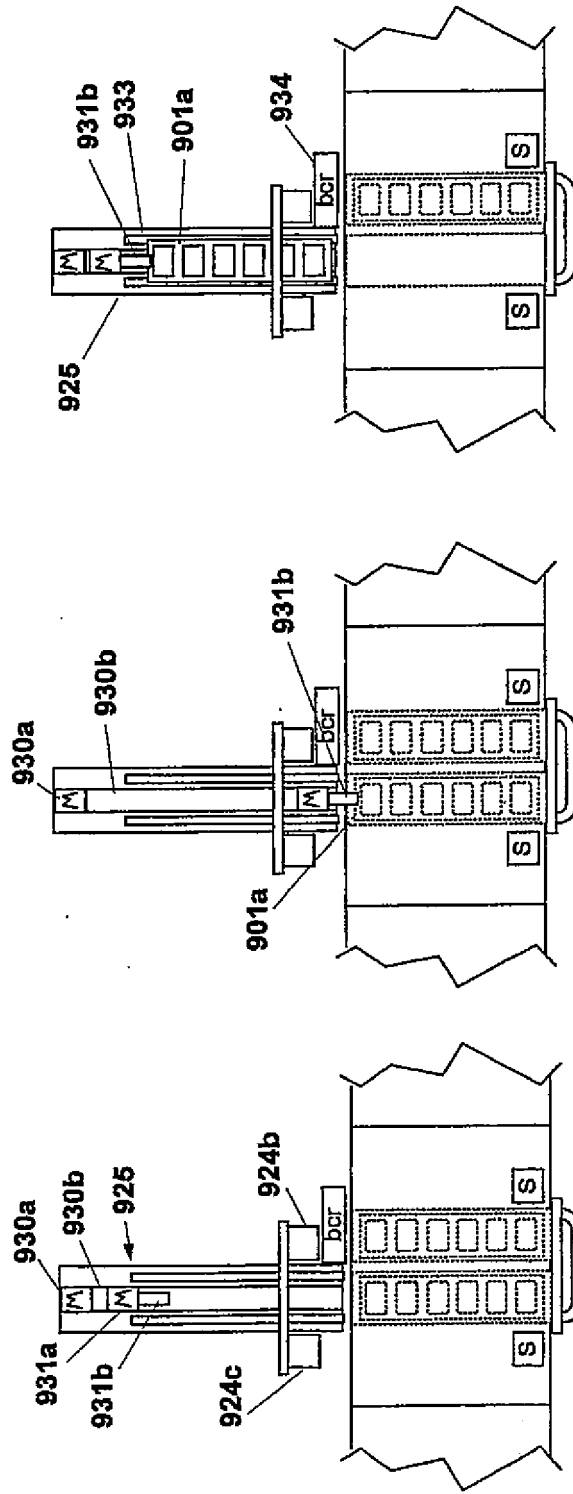












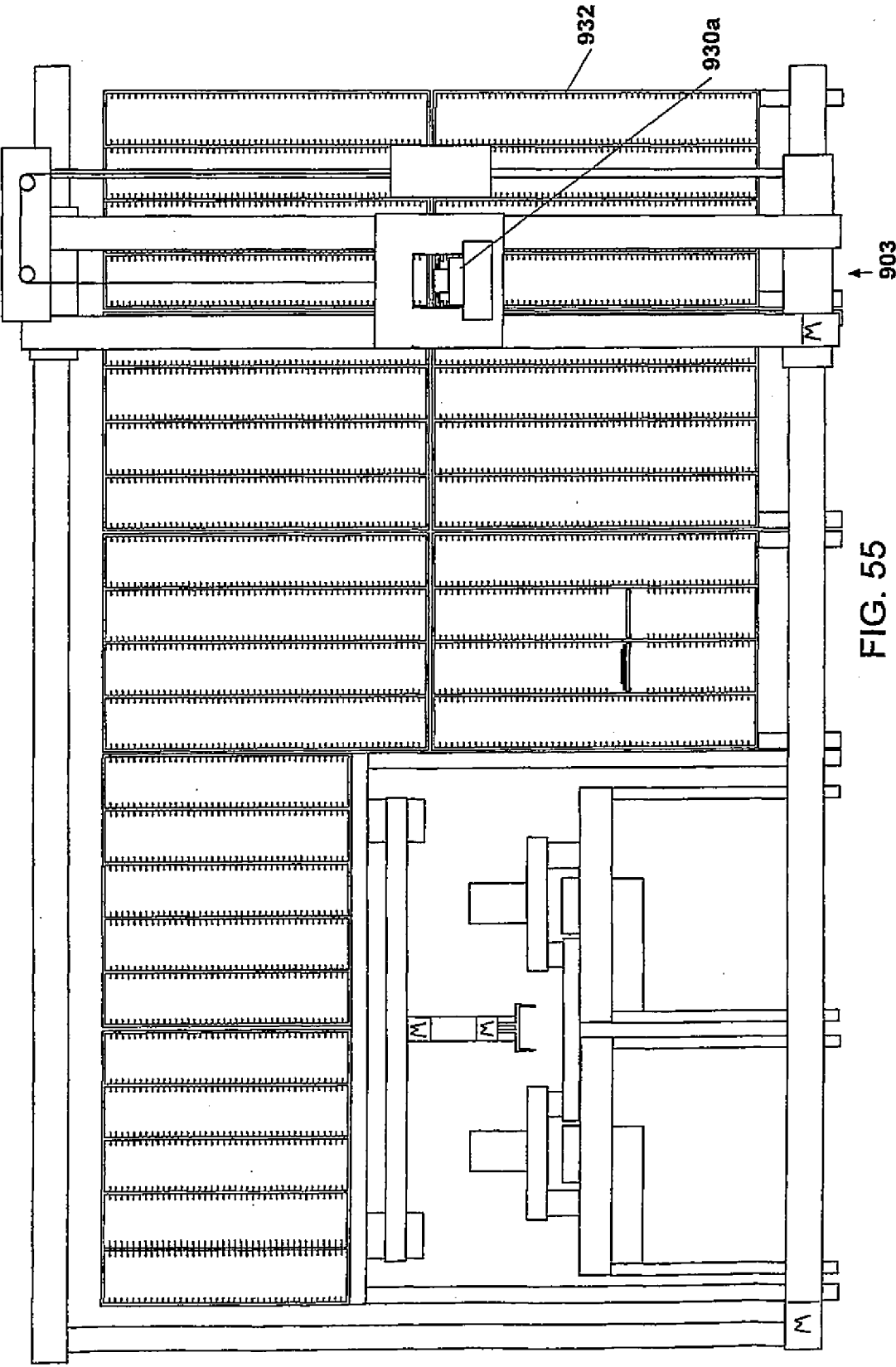
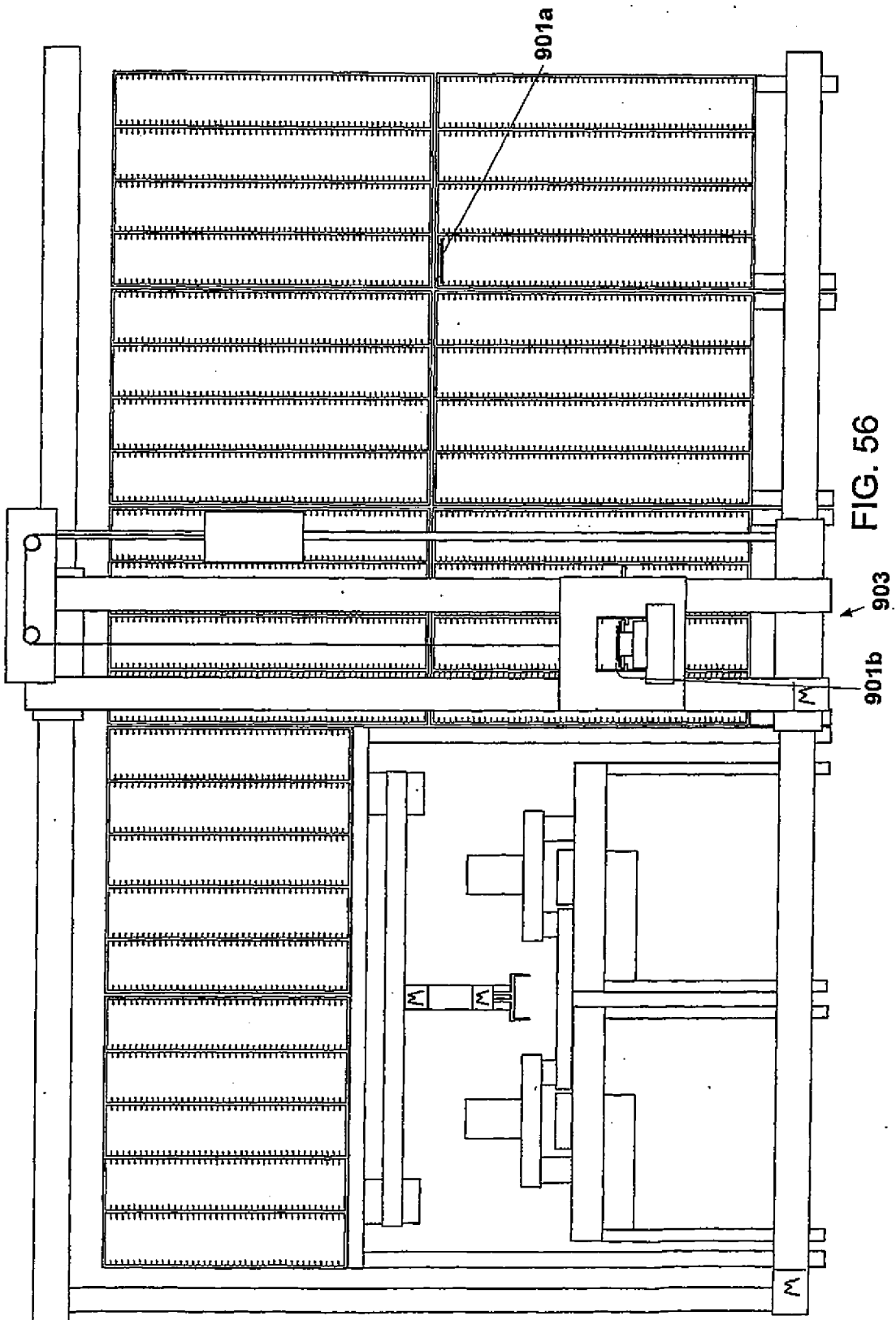
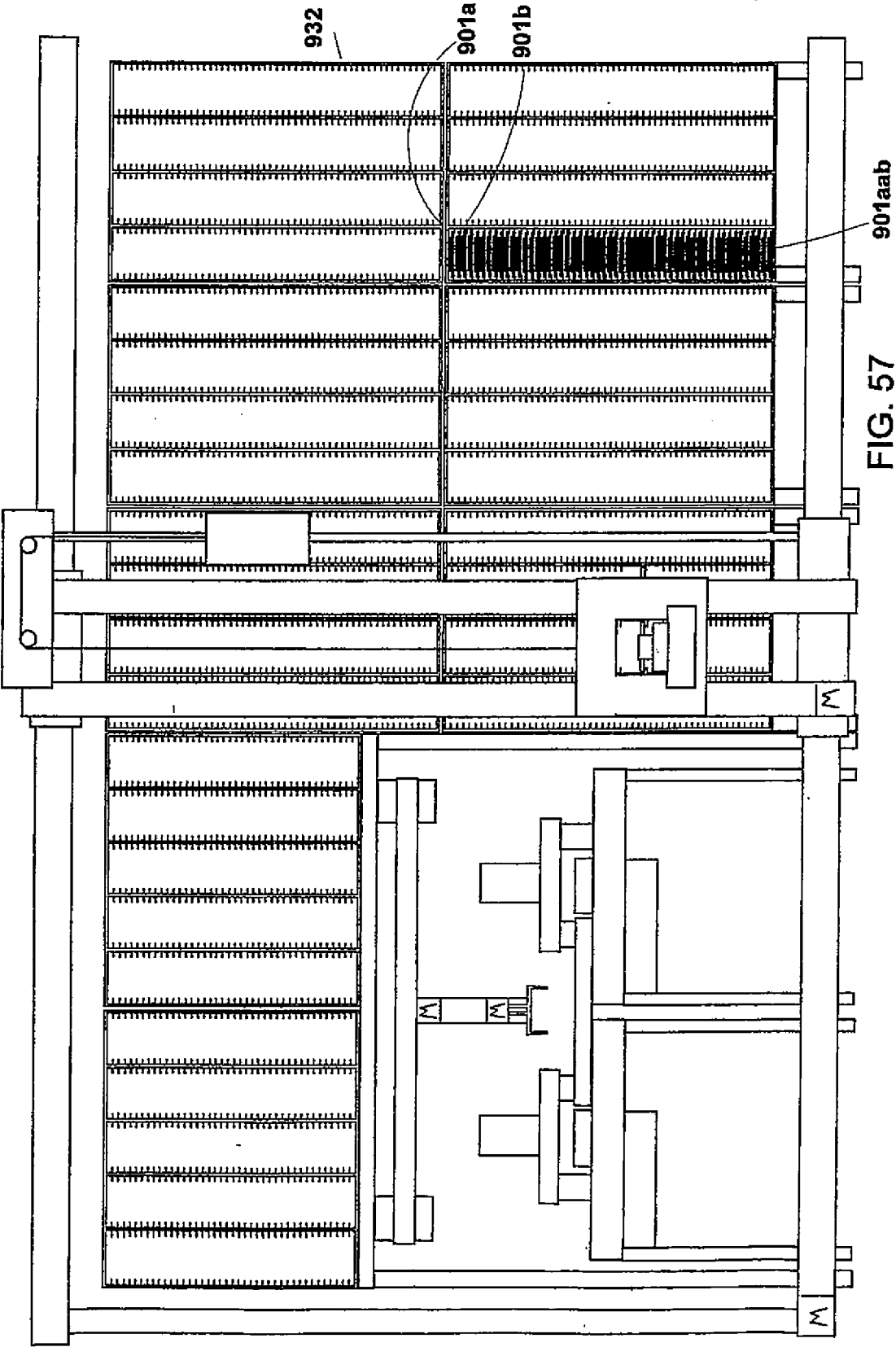
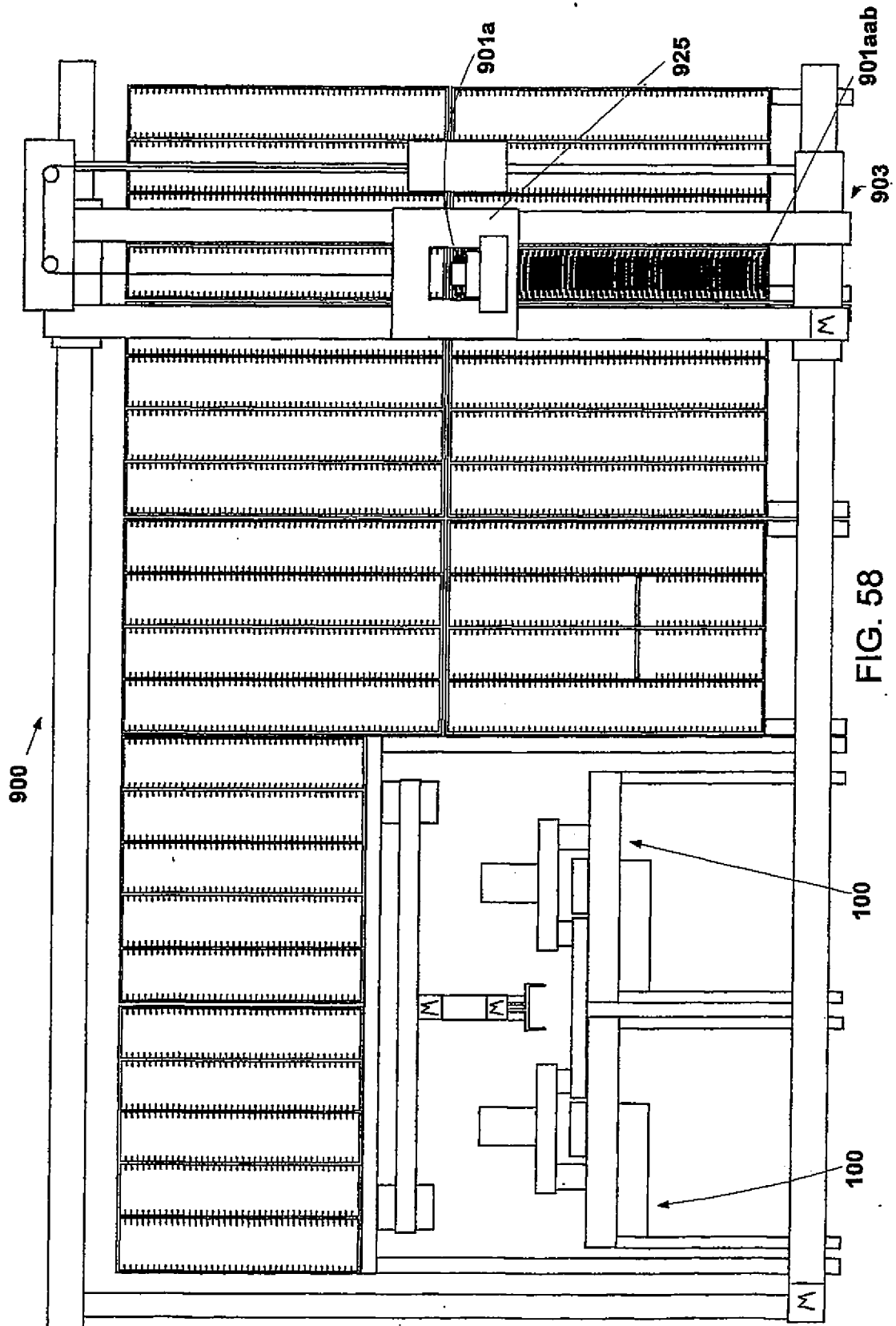
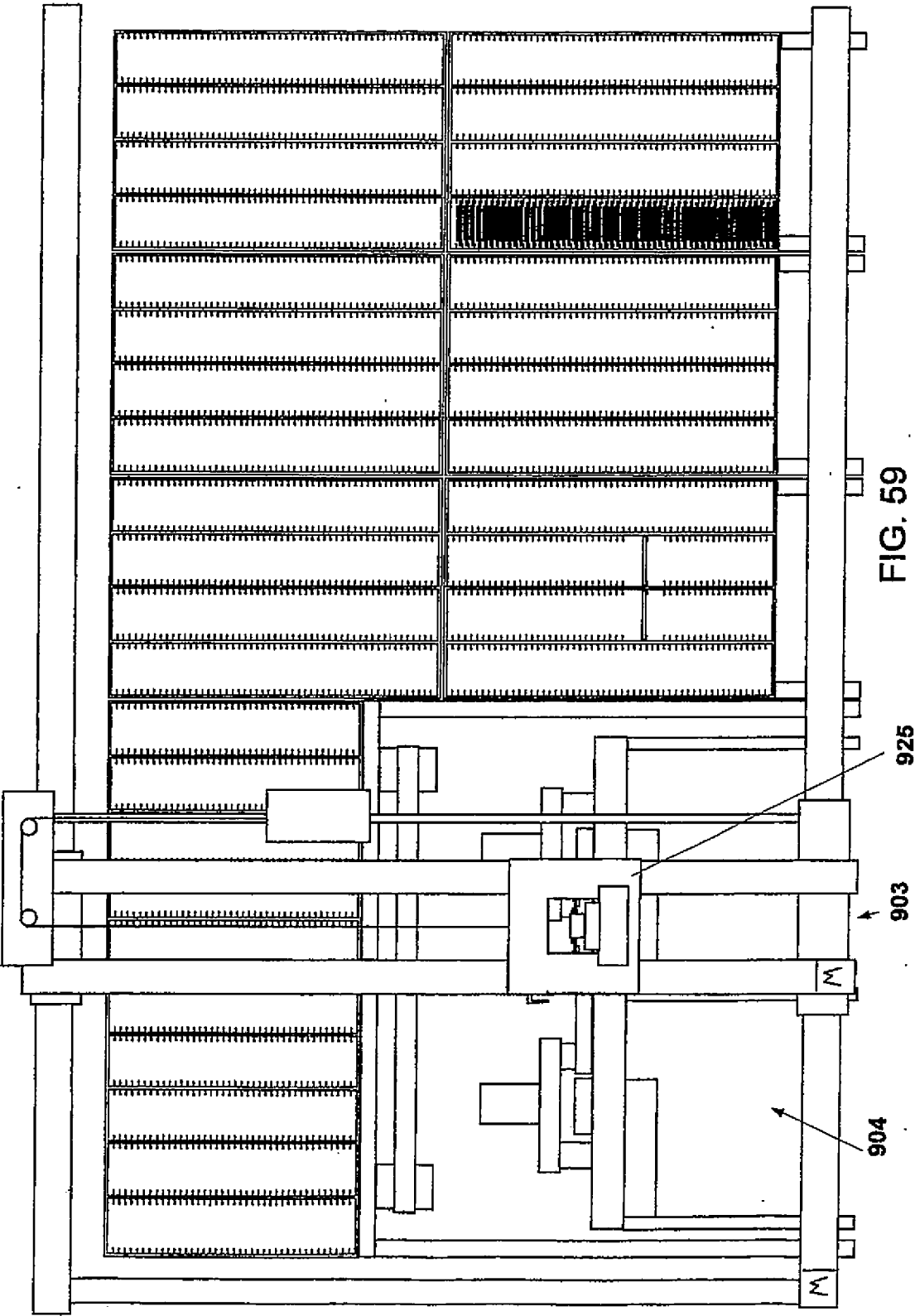


FIG. 55

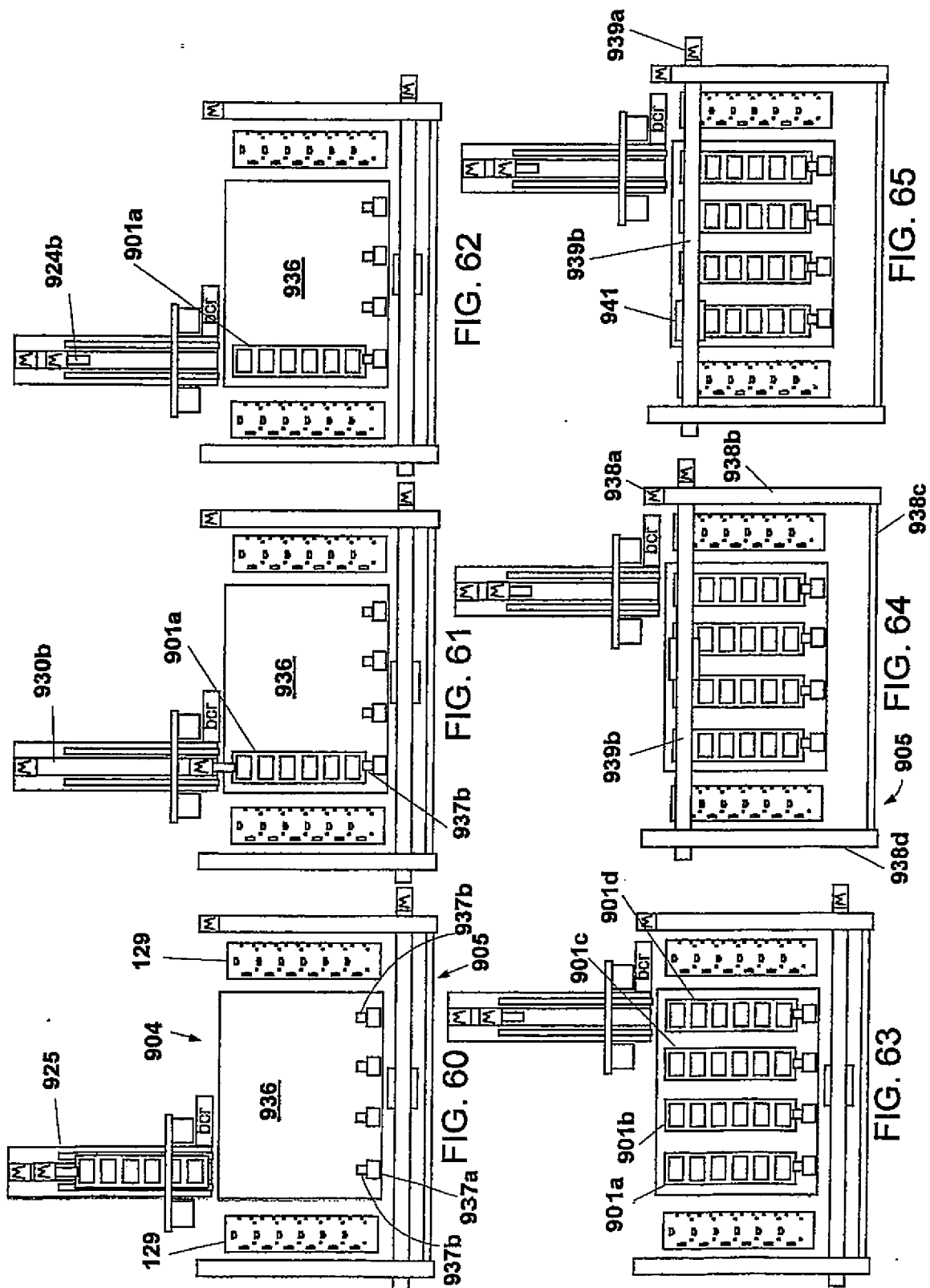












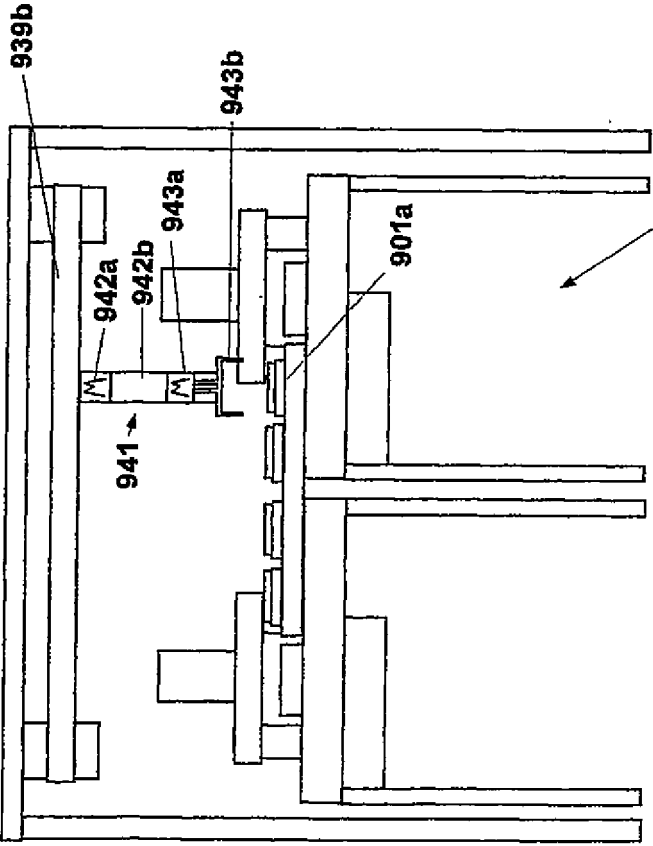


FIG. 66

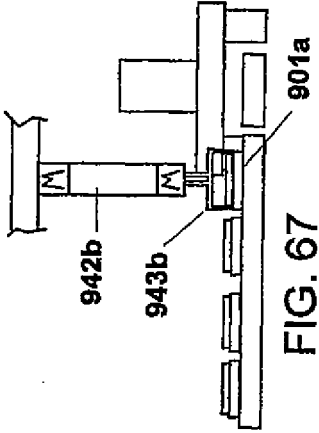


FIG. 67

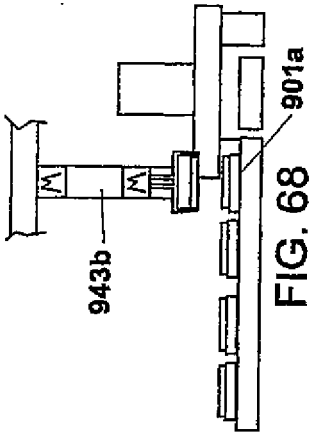


FIG. 68

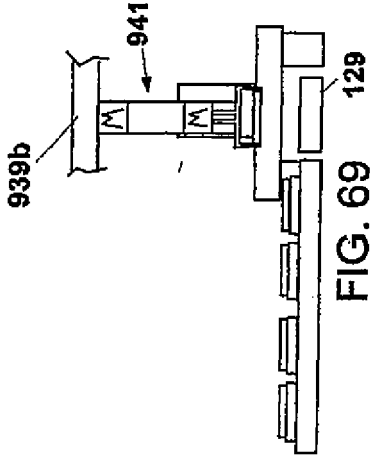


FIG. 69

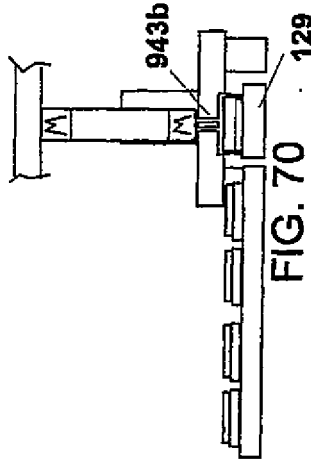


FIG. 70

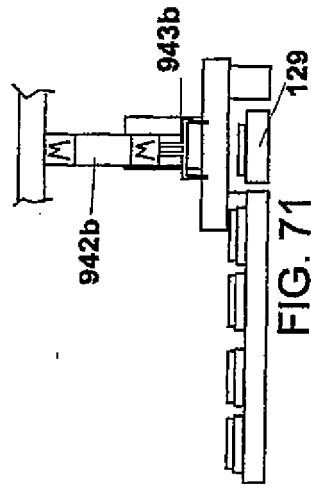


FIG. 71

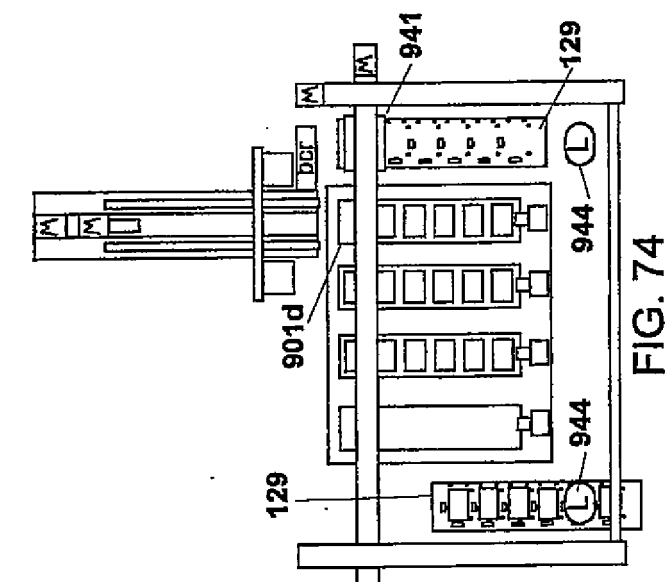
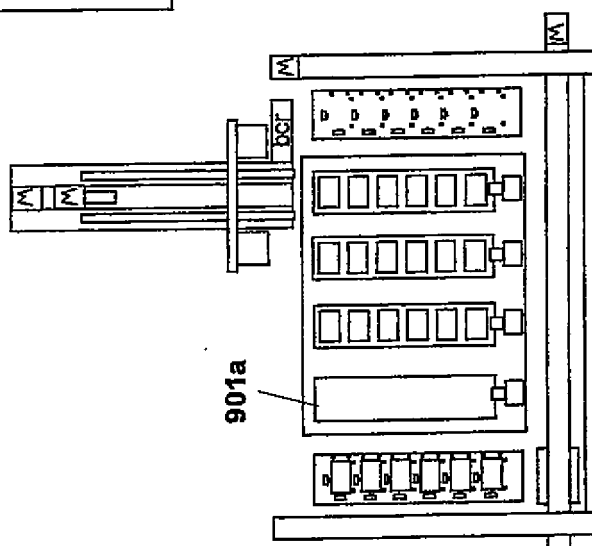
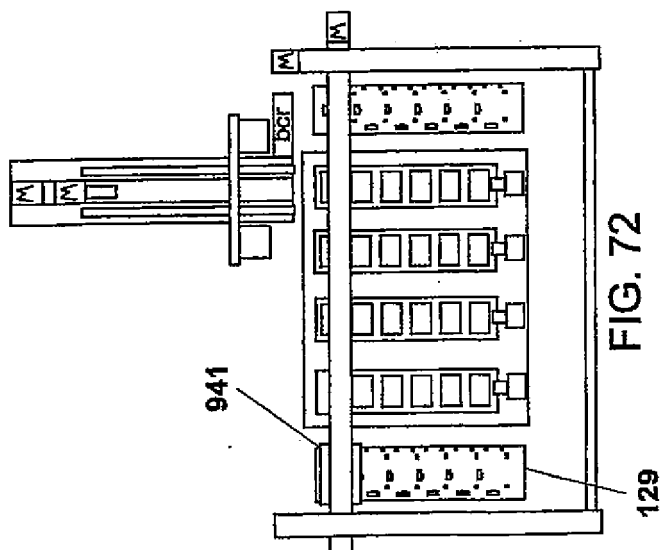


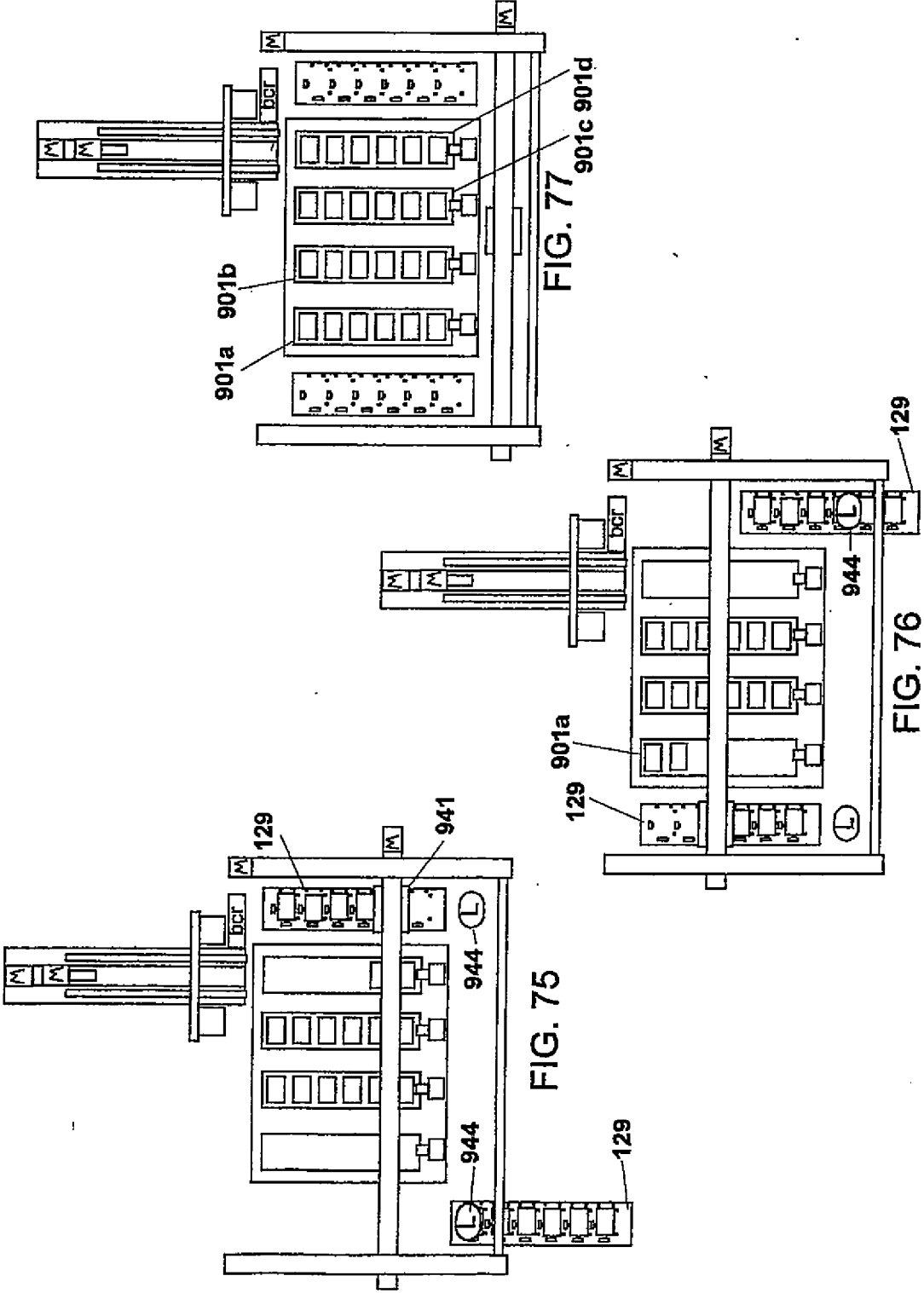
FIG. 74

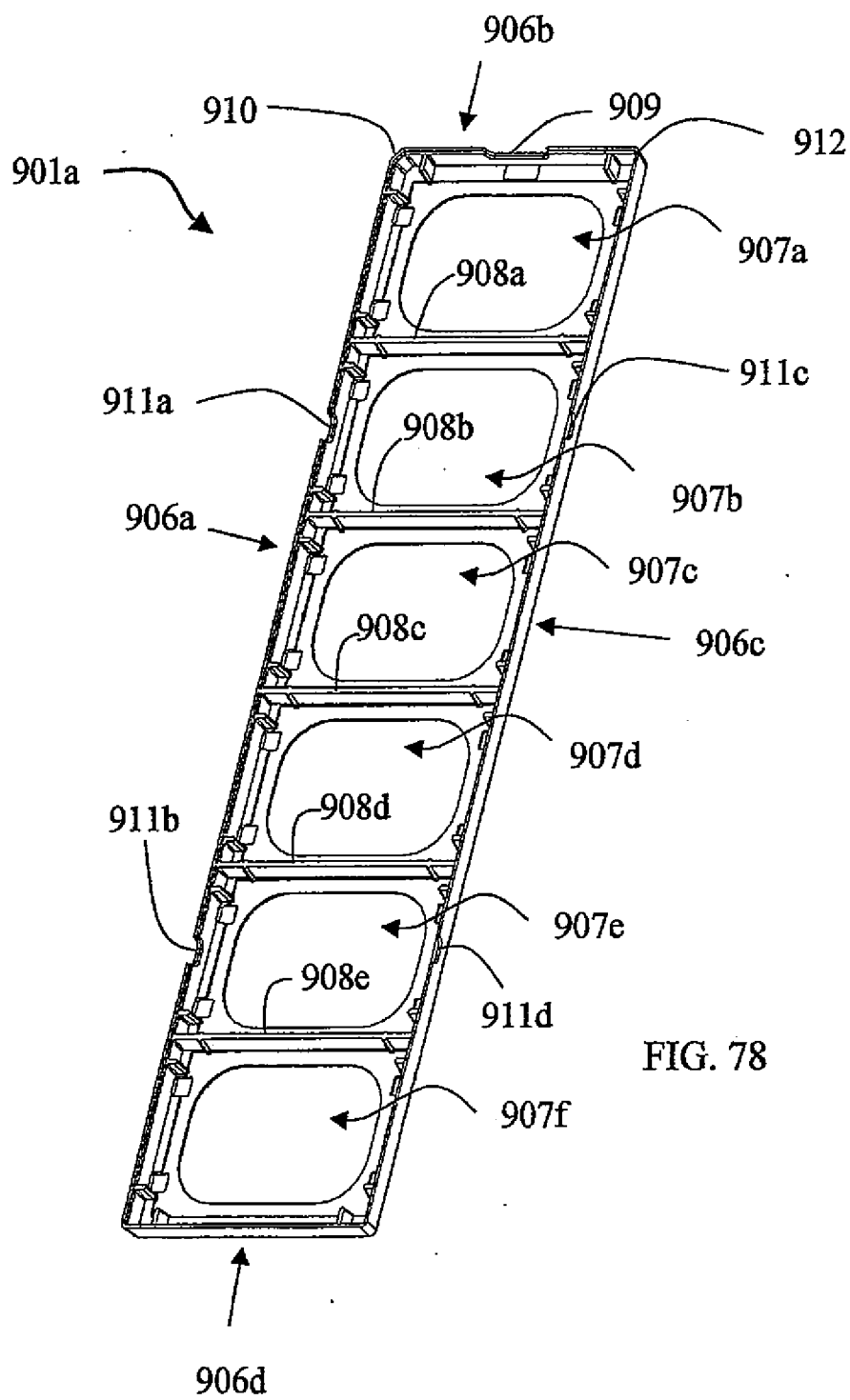


**FIG. 73**



**FIG. 72**





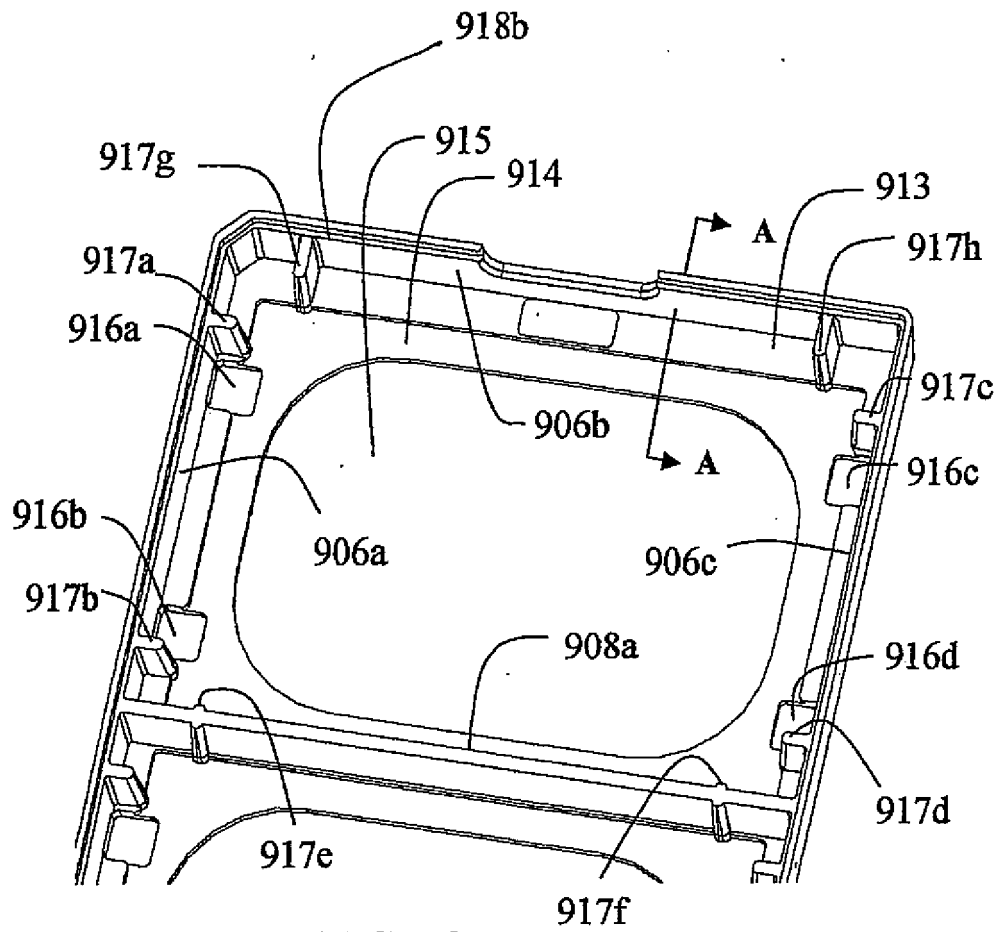
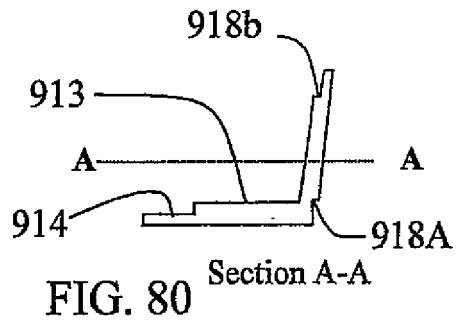


FIG. 79

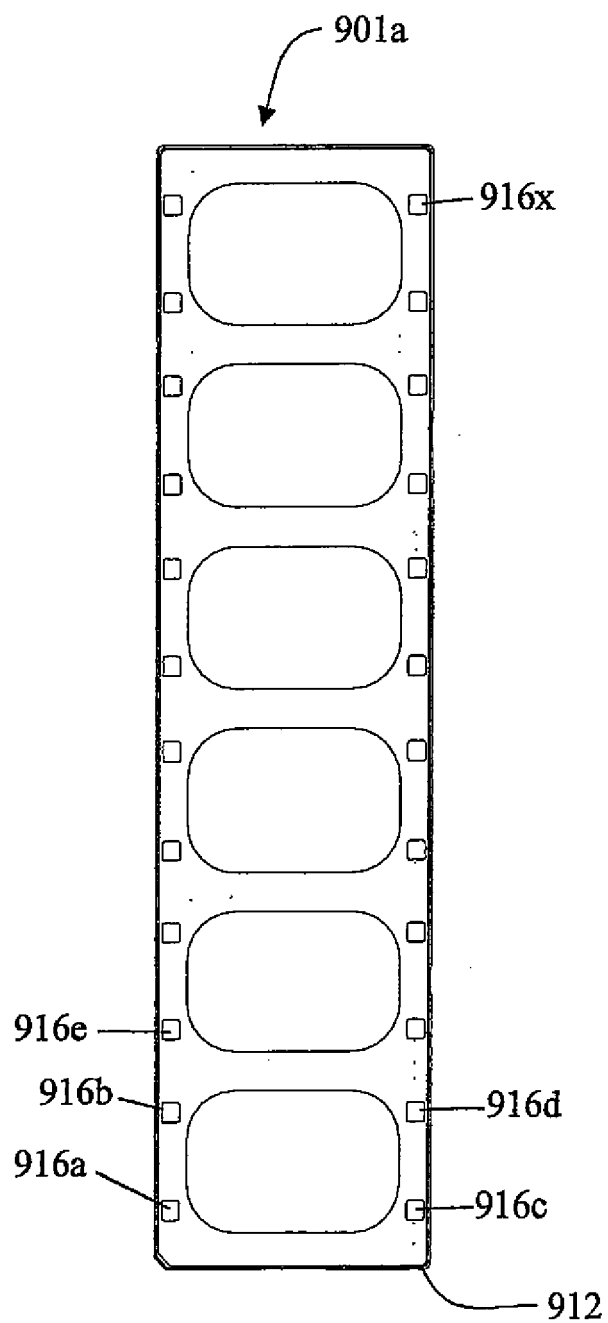


FIG. 81

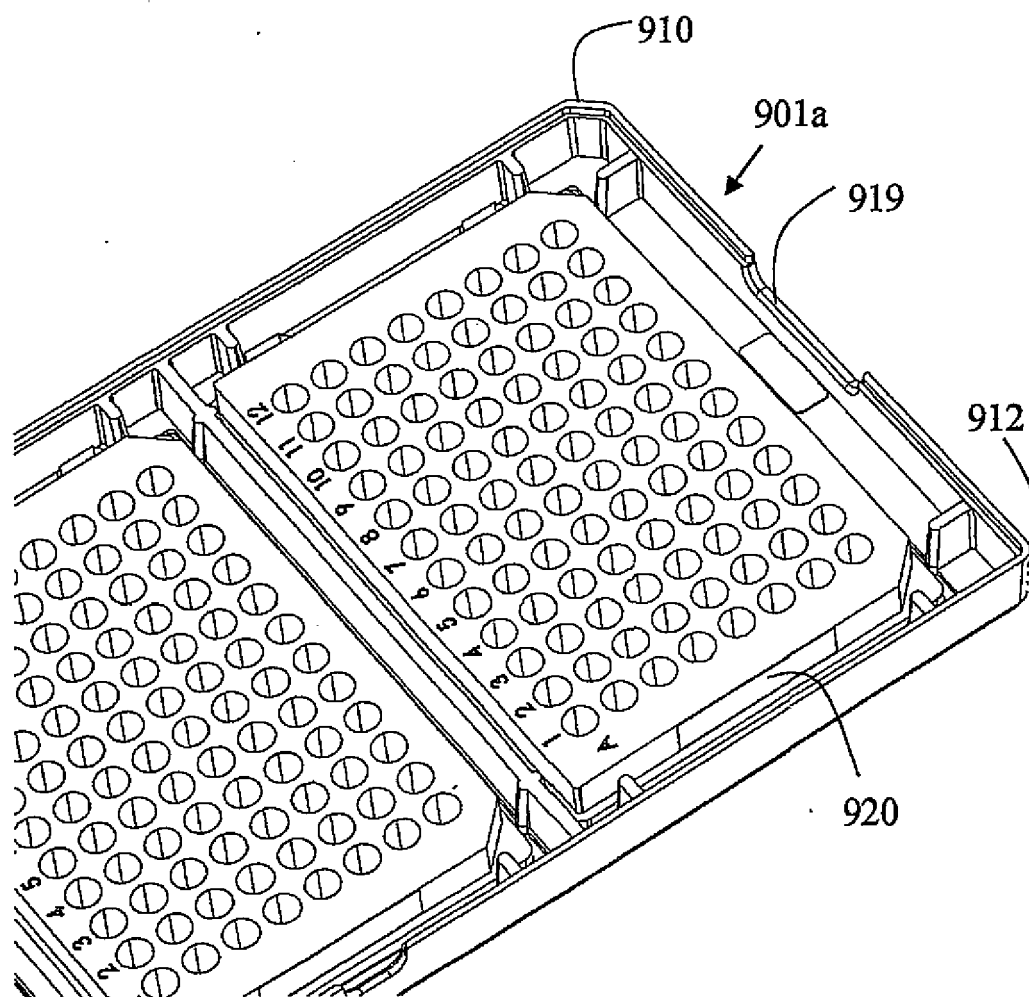


FIG. 82



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/02107

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : B01L 3/02

US CL : 141/130; 422/100;

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 141/130; 422/100,99; 435/286.4,287.3; 347/19,85

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
EAST

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,001,309 A (GAMBLE et al.) 14 December 1999 (14.12.1999), see entire document.	1-34
A	US 5,988,236 A (FAWCETT) 23 November 1999 (23.11.1999), see entire document.	1-34
A	US 5,865,224 A (ALLY et al.) 02 February 1999 (02.02.1999), see entire document.	1-34

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

26 March 2003 (26.03.2003)

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20 MAY 2003

Name and mailing address of the ISA/US

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